

THE  
BOTANICAL GAZETTE

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EDITOR  
JOHN MERLE COULTER

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WITH FOURTEEN PLATES AND ONE HUNDRED AND SIXTY-THREE FIGURES



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DATES OF PUBLICATION

No. 1, July 16; No. 2, August 14; No. 3, September 17; No. 4, October 15; No. 5, November 15; No. 6, December 18.

#### ERRATA

- P. 53, line 14 from top, begin new sentence with Vexillum.
- P. 58, line 10 from top, for Comarea read Comarca.
- P. 60, first line, for glandula read glandulae.
- P. 60, line 5 from top, for purpurascences read purpurascentes.
- P. 184, last line, for solutions read solution.
- Pp. 189 and 191, exchange legends of fig. 8 and fig. 9.
- P. 261, line 18 from top, for fungus read fungous.
- Pp. 289, 290, 293, tables VI, VII, VIII, last column, for (total control) read (total-control).



THE  
BOTANICAL GAZETTE

JULY 1913

## THE LIFE HISTORY OF ZANARDINIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 174

SHIGÉO YAMANOUCHI

(WITH TWENTY-FOUR FIGURES AND PLATES I-IV)

This paper deals with nuclear conditions in the life history of *Zanardinia collaris* Crouan, a monotypic genus which with the genus *Cutleria* constitutes the Cutleriaceae. *Zanardinia* is perennial and, like *Cutleria*, is characterized by large motile spores, whose formation is limited to a very short period of the year. On account of the shortness of this period and the consequent difficulty in getting reproductive stages, it was described as a species of *Zonaria* (AGARDH 1) belonging to the Dictyotales, but was placed in the Cutleriaceae under its present name when CROUAN discovered that it had gametangia identical with those of *Cutleria*.

Since AGARDH (1), several authors, including DERBÈS and SOLIER (7), CROUAN (4), JANCZEWSKI (11), and SAUVAGEAU (15), have described the outer morphology of the creeping and coriaceous thallus of *Zanardinia* and its multiplication by proliferation from the surface; and it must be admitted that some of these authors confused the young *Zanardinia* with immature stages of *Cutleria adspersa*, the two forms being confusingly alike in the juvenile condition.

REINKE (13, 14) was the first to describe the reproductive structures of *Zanardinia*. He observed, working at the Naples Station in 1875-1876, the actual fertilization of the female gametes by the male gametes. There seemed to be no apogamous germina-

tion of the unfertilized eggs. From the sporelings of both fertilized gametes and zoospores he obtained the filaments of *Zanardinia* which directly produce non-motile spores (*Secundärsporen*), a single one in each cell. In his cultures *Desmotrichum* appeared later, and consequently the ultimate fate of the sporelings and of the non-motile spores was not clearly determined.

SAUVAGEAU (15) is one of those who have done most work on the Cutleriaceae, having been publishing the results of cultures of *Cutleria* since 1898. He has shown that the thallus of *Zanardinia* is formed through the union of marginal filaments, and has described in detail the dorsiventral structure and the general topography of the constituent cells.

The first cytological paper was that of the writer, which was published as a preliminary note two years ago (21). The material was collected in the Bay of Naples in the winter of 1908 and the spring of 1909, during which time I occupied a table of the Carnegie Institution at the Stazione Zoologica. *Zanardinia* was found in the vicinity of Posilipo growing on the surface of rocks or sunken wooden blocks down to a depth of about 25 meters. Cultures of the plants and of their sporelings and fixation of critical stages were made in the laboratory of the Station. The study thus begun at Naples was finished at the Hull Botanical Laboratory of the University of Chicago.

The paper presents first the mitosis in the vegetative cells of the gamete-bearing plants, the formation of the gametes, the fertilization and germination of the fertilized female gametes, and the apogamous germination of unfertilized female gametes; then there is described the mitosis in the vegetative cells of the zoospore-bearing plants, the formation and germination of the zoospores; and finally, there is a brief statement concerning an alternation of generations in the life history of *Zanardinia*.

#### Mitosis in the vegetative cells of the gamete-producing plants

Gamete-producing plants of *Zanardinia* in early stages of development, while in the form of a concave disk or cup resembling *Peziza* and no larger than 1 cm. in diameter, showed numerous mitotic figures in the superficial layers of the thallus, no matter

what time of day or night the material was fixed. After the plant became 5 cm. or more in diameter, mitosis was much more frequent near the margin of the thallus or in hairs growing from the margin. After the plant reaches the adult size (8-10 cm. in diameter), the figures are very rare in the inner tissue and only occasionally found in superficial layers.

The cells of *Zanardinia* have quite thick walls, which are thickest in the huge inner cells and less thickened in the cells of the superficial layers that contain more numerous plastids. The plastids take stains with avidity, and it was more difficult to bring out the details of the mitotic figure than in *Culleria* and *Fucus*. The nuclei in the resting stage, in almost any part of the thallus, are all about the same size and are a little larger than the plastids. Each contains a small, deeply staining nucleolus, which lies in the center. The remainder of the nuclear cavity is almost wholly occupied by a large body of karyolymph, and a few scattered chromatin granules lining the nuclear membrane.

One of the conspicuous features of the resting nucleus of *Zanardinia* is the fact that outside the membrane there are frequently a number of deeply staining globules of irregular size. These globules are so close to the membrane that for some time I was in doubt whether they were within or without the membrane. Careful study, however, showed clearly that certain of them are outside the membrane and yet in close contact with it (fig. 1). As the nucleus increases in size and the chromatin granules within increase in quantity, delicate chromatin fibrils of irregular size appear among the granules. During this increase of chromatin granules, the deeply staining globules outside the membrane decrease, and finally disappear. A comparative study of many such cases has convinced me that the globules are quite closely allied to the chromatin and seem to pass readily through the nuclear membrane.

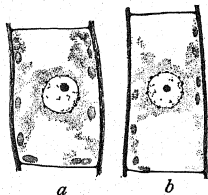


FIG. 1.—Portions of vegetative filaments: *a*, of zoospore-bearing plant; *b*, of gamete-bearing plant; nucleus with chromatic accumulations along the outside of the membrane.

Chromatin granules thus formed gradually move away from the membrane; the chromatin fibrils lying mixed with the granules form globules here and there; and the granules and globules then develop so that they become nearly spherical and uniform in size, being about 22 in number. They are chromosome primordia. The chromatin fibrils are all used up in the formation of chromosomes, and the nucleus contains only the chromosomes, a nucleolus, and the nuclear sap. When the chromosomes become arranged at the equatorial plate, kinoplasmic accumulations, developed from the cytoplasm surrounding the nuclear membrane, appear at the poles. A well marked centrosome-like structure in the kinoplasmic masses occurs only at metaphase, disappearing in anaphase. The chromosomes split longitudinally and half of each chromosome proceeds to each pole. During this process, the spindle is intranuclear. At telophase or at late anaphase, the nuclear membrane disappears and the two sets of crowded daughter chromosomes are surrounded by the cytoplasm, and the formation of the nuclear membranes follows. When the daughter nuclei are organized, the central spindle disappears completely. The cytoplasm lying between the two nuclei assumes a coarse, irregular alveolar structure, and the walls of the alveoli, probably after a change in their material, form a new cell plate. This process is similar to that in *Fucus* (20) and *Cutleria* (22).

#### Male and female gametangia

*Mature gametangia.*—Both male and female gametangia occur mixed on certain parts of the surface of the thallus (fig. 2). When the mature plant is living and fresh, the parts where the gametangia are borne are conspicuous from the deep dark color, as distinct from the deep brown of the general sterile surface. Both male and female gametangia are non-branched filamentous structures arising from superficial cells of the thallus. The male gametangia mature earlier than the female in the same individual. The upper surface of the plant, except the margin, is smooth and is devoid of any hairy growth. The patches of sori are composed exclusively of filaments ending in terminal gametangia. Occasionally, however, mixed with the ordinary gametangia are hairs which



in their middle region produce male or female gametangia, indicating that the latter are hairs in origin.

The mature male gametangium consists of a number of tiers of small cells (the male gamete mother cells), each tier comprising 8 cells, and since there are at most 33 tiers (fig. 4, *b*), the output of a single male gametangium is about 264 gametes. The mature male

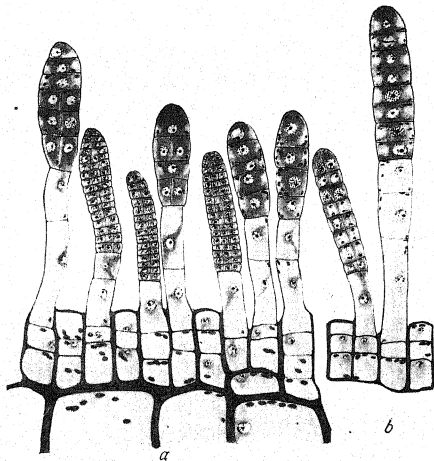


FIG. 2.—Portions of thallus with a number of filaments bearing both male and female gametangia: *a*, male and female gametangia with stalks composed of one or two cells; most of them are near maturity; *b*, young gametangia; a female gametangium with a stalk of three cells.

gamete in the free swimming condition outside the gametangium is oval and usually contains two plastids. A portion of one of the plastids lying laterally near the anterior end has a deep orange color, which is the red pigment, and in close association with this pigment are two cilia, one directed toward the anterior end, 6 times the length of the gamete, and the other in the opposite

direction, about 2.5 times the length of the gamete. The length of the entire male gamete is  $4.5 \mu$  (fig. 16, b).

*Development of the male gametangia.*—As stated above, male gametangia arise from superficial cells of the thallus. One of the

superficial cells divides, giving rise to a gametangium initial and a stalk cell. Often two or more subsequent divisions occur and there is produced a filament of two or more cells, the terminal one of which is a male gametangium initial (fig. 5, a, b, c). In rare cases a filament becomes a long multicellular hair consisting of a single row of cells, and some cell in the middle of the hair becomes the gametangium initial. This shows that the male gametangium is a hair in origin (fig. 3, c, d). This development of a male gametangium from a single superficial cell, occurring simultaneously in multitudes of neighboring cells on large areas of the surface of the thallus, results in the production of thousands of gametangia growing side by side, producing the dark-colored patches upon the thallus.

The nucleus of the male gametangium initial increases considerably in size. The chromatin network of the resting nucleus is marked by a number of knots mixed with fibrils. The chromatin knots increase gradually in size in prophase and finally break up into 22 chromosomes. When chro-

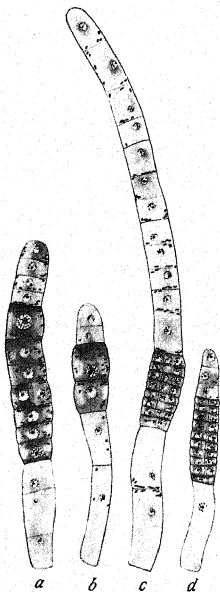


FIG. 3.—Filaments bearing gametangia in their middle region: a and b, with female gametangia; c and d, with male gametangia.

mosomes become arranged at the equatorial plate, two distinct centrosome-like structures are present at the poles. The nuclear membrane either persists or disappears at metaphase (fig. 6, a, b).

The nucleus passes into telophase, two new daughter nuclei are formed, and then the subsequent divisions occur. The details were followed from the second division to the last, and the processes are simply a repetition of the first. When the young gametangium has reached the stage consisting of a single row of 7 or more cells, there occur also divisions perpendicular to the axis of the gametangia, so that each tier of the gametangium consists of 2 (fig. 6, *i*), 4 (fig. 6, *j*), or 8 cells (fig. 7, *a*). During these divisions the nuclei in the gametangium do not necessarily divide simultaneously, but often in quite irregular order (fig. 6, *c-g*). The size of the nuclei does not diminish in spite of the gradual and considerable diminution in size of the cell (fig. 6, *a-j*); for example, the figure in the first division in the gametangium (fig. 6, *a*) and that of the last division (fig. 6, *g, j*) are alike in size, while the first cell is ten times as large as the last.

During these mitoses in the gametangia, chromosomes were counted in prophase, in polar view of metaphase and anaphase, and the number is 22.

Regularity in the axes of these divisions, which take place either parallel or perpendicular to one another, results in producing the well known male gametangia of *Zanardinia*, comprising more than 30 tiers, each tier made up of 8 cells (fig. 7, *b*). Each individual cell in the gametangium is a male gamete mother cell, within which a single male gamete is formed. The mother cell contains a single large nucleus situated in the center, and usually two plastids whose position varies (fig. 7, *a, b*). The nucleus passes into a complete resting condition. One of the plastids moves near the nucleus and then a part of the plastid body becomes deep orange in color, which is the red pigment (fig. 7, *c*). When

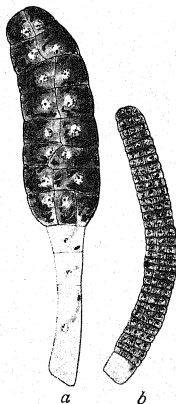


FIG. 4.—Mature gametangia: *a*, female gametangium with 8 tiers of gamete mother cells; *b*, male gametangium, with 33 tiers of gamete mother cells.

the male gamete is mature, a portion of the free surface of the membrane of the mother cell dissolves so as to leave a pore, through which the gamete is discharged (fig. 8). The cilia of the gamete first protrude from the pore, keep moving for a while, and then the whole body of the gamete emerges and is set free.

*Mature female gametangia.*—Mixed with male gametangia, there are developed female gametangia, which arise like the former from superficial cells of the thallus. The mature female gametangium consists of several tiers of mother cells, the number of tiers varying from 3 to 9 (figs. 2 and 4, *a*). Each tier comprises 2 or 4 mother cells, so that the output of gametes from a single female gametangium fluctuates between 6 and 36. The mature female gamete in

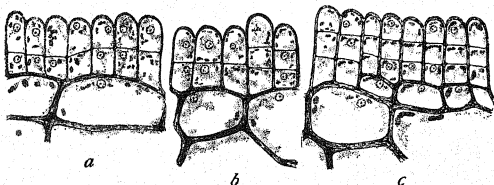


FIG. 5.—Portions of thallus showing origin of male gametangium initial: *a*, *b*, *c*, 2, 2 or 3, and 3 or 4 layers of superficial cells respectively, outermost of which in each case are male gametangia.

the free swimming condition outside the gametangium is oval (fig. 16, *a*) and usually contains less than 30 plastids. The anterior end of the body is destitute of plastids and consists of colorless granular cytoplasm, thus indicating polarity in the organization of the body. A portion of some plastid near the periphery in the anterior end takes up a deep orange coloring matter, which is the red pigment. Close to the pigment, two cilia are borne; one being directed toward the anterior end, 2.25 times the long diameter of the gamete, and the other in the opposite direction, 1.3 times the diameter of the gamete. The diameter of the female gamete is 19–23  $\mu$ . Active motility of the female gamete does not last long; at the longest under observation, the movement becomes sluggish within an hour, the shape becomes spherical (figs. 16, *c*, and 26), and the cilia are withdrawn or coalesce with the protoplast.

*Development of female gametangia.*—Like the male gametangium, the female gametangium arises from a superficial cell of the thallus. One of the superficial cells divides, giving rise to a gametangium initial and a stalk cell. A second or third division may be intercalated between the first division of the superficial cell and the

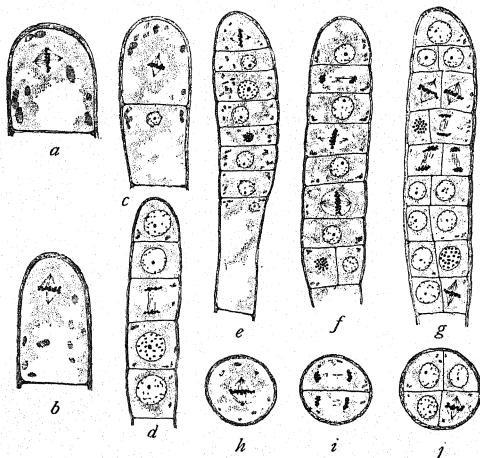


FIG. 6.—Male gametangia in various stages of development: mitotic figures in prophase, metaphase, and anaphase show 22 chromosomes: *a*, one of the superficial cells, whose division will result in two gametangium initials; *b*, first division in a gametangium initial; *c*, 2-celled stage; *d*, 5-celled stage; *e*, 7-celled stage; *f*, gametangium comprising 8 tiers; *g*, comprising 9 tiers; *h*, *i*, and *j* show respectively the cross section of gametangia of 1, 2, and 4 rows of cells.

differentiation of the gametangium initial, and in this case, the female gametangium has a stalk consisting of two or more cells (figs. 1 and 9, *a*, *b*). In rare cases, the female gametangium initial develops from a middle cell of a multicellular hair which has arisen from a superficial cell, indicating the hair origin of the female gametangia. The formation of the female gametangium takes place

simultaneously in multitudes of neighboring superficial cells, so that thousands of filamentous gametangia are produced, covering a considerable area of the surface of the thallus.

The nucleus of the female gametangium initial increases greatly in size, like that of the male, and becomes larger than the vegetative nucleus. The chromatin of the nuclear reticulum gradually increases and finally there is organized a prophase with 22 chromo-

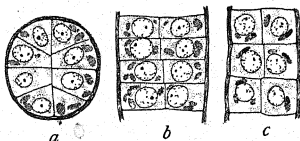


FIG. 7.—Mature male gametangia: *a*, cross section; *b*, tangential longitudinal section; *c*, optical section viewed laterally, in which red pigment spots have developed in a part of the plastids closely associated with each nucleus.

somes (fig. 10, *a*) and a single nucleolus. The nuclear membrane either persists or disappears at the time of the organization of the equatorial plate (fig. 10, *b*, *c*). The centrosome-like structures at the poles are present only during metaphase and early anaphase. At telo-

phase, two new daughter nuclei are formed, and no central spindle remains between them. The cell plate is laid down by the cooperation of the vacuoles and by the transformation of cytoplasm.

The gametangium initial in the unicellular stage, whether male or female, shows no difference so far as the visible structure and size are concerned. The marked difference in the size of the male and female gametangia begins when the gametangia have reached the two-celled stage (figs. 6, *c*, and 11, *b*). The considerable growth of the cells in the gametangium is accompanied by that of the nuclei, and the details of the nuclear division are more readily and accurately followed during the gradual development of the female gametangium. As in the case of the male gametangium, subsequent divisions were followed up to the last division (figs. 11–15). The nuclear divisions in the cells do not take place simultaneously. When the gametangium has attained the three-celled stage, a cell in each tier divides transversely. One more transverse division may or may not occur; in the former case each tier consists of 4 cells (fig. 15, *b*), and in the latter it remains composed of 2 cells (fig. 15, *c*).

During these mitoses, chromatin globules accumulating outside of the membrane of the resting nucleus, and their gradual disappearance with the simultaneous increase of chromatin substances within the network, are beautifully shown. In prophase, in metaphase viewed from the pole, and in anaphase, 22 chromosomes are distinctly shown (figs. 11-14) proceeding toward the pole with equal rapidity.

The female gametangium comprising 3-9 tiers of cells, with 2 or 4 cells in each tier, is now established. Each individual cell of the gametangium is a mother cell and the whole contents of the cell become transformed into a single female gamete. The mother cell contains a large resting nucleus, surrounded by plastids. A portion of one of the plastids lying near the nucleus shows a deep orange color, which is the red pigment (fig. 14, *a, b, c*). When the female gametes are matured, a portion of the membrane of the mother cell dissolves, forming a pore through which the gamete is discharged. The cilia of the gamete first appear outside the pore, keep waving for a time, and then the gamete is set free.



FIG. 8.—Mature male gametangium after escape of gametes.

#### Fertilization and germination of the fertilized female gamete

The discharge of both male and female gametes occurs at almost any time during the day and night. Taking the case of a certain individual plant growing in a tank in the laboratory, the male gametangia matured and the discharge of the gametes began while the female gametangia were still in an immature condition, and did not mature and discharge their gametes until two or three days later. The time relations of the maturity of the male and female gametes in nature may be similar to that in artificial cultures. A periodic discharge of the gametes from the mature gametangia was observed to be most abundant at about 5:30 A.M., after which the discharge

gradually diminished, and finally ceased about 8:00 A.M. The male gametes continue in the motile condition for more than 24 hours, while the female gametes retain their motility for scarcely one hour and sometimes only for a few seconds. Toward the end of the motile condition, the movement of the gametes becomes sluggish, the cilia become coalescent with the protoplast, and finally the shape of the gametes becomes spherical. Even after the gametes have assumed the spherical form the polar organization in regard to the distribution of plastids is not lost. The area of granular cytoplasm with no plastids remains for a considerable time. From observation of the living material, it is evident that the formation of the wall, its

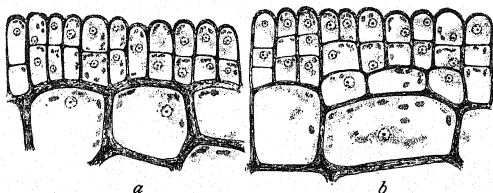


FIG. 9.—Portions of thallus showing the origin of the female gametangium initials: *a* and *b*, 2 and 3 layers of superficial cells respectively, outermost of which in each case are female gametangium initials.

subsequent thickening, and the elongation of the sporeling, both of fertilized and unfertilized female gametes, are in this area of the anterior end of the gamete. The union of male and female gametes and the succeeding nuclear behavior were studied in the material from artificial cultures as in my investigation of *Cutleria* (22). The fixations of the sporelings were made every 30 minutes for 24 hours, and then at 30, 36, 40, 46, 48 hours, and later every 5 days up to 30 days.

The male gametes while swimming freely become attached to the female gametes which are moving actively or sluggishly. Numerous male gametes, however, after swimming for a long period, even 24 hours, fail to come into contact with the female gametes, and then the movement ceases and the cilia fuse with the plasma



membrane of the body, which now becomes spherical. The nucleus, with a very delicate membrane, shows a number of chromatin knots identical with the number of chromosomes (fig. 25). It is a noticeable fact that the nucleus of the male gamete, during the period of active movement, is in the resting condition, and when the gamete becomes quiescent without any union with a female gamete, it still shows the same structure as if it had united with the female gamete.

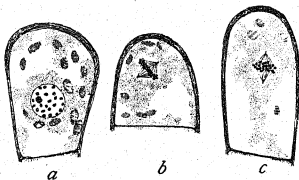


FIG. 10.—Female gametangia: nuclei in the first division.

When the male gamete has just become attached to the female gamete, both gametes have very delicate plasma membranes.

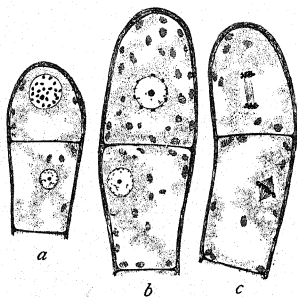


FIG. 11.—Female gametangia in 2-celled stage: *a*, nucleus in prophase, showing 22 chromosomes; *b*, nucleus with the accumulation of chromatin globules around the outside of the membrane; *c*, one nucleus in anaphase and the other in metaphase.

The nucleus of the female gamete is at the center of the cell, as in the resting condition, but that of the male gamete shows 22 independent chromosomes. The plasma membranes which lie between the cytoplasm of the male and female gametes become obscure and the cytoplasm of the two gametes comes into direct contact (fig. 27). The body of the male gamete can be observed for a short period as a protuberance from that of the female gamete (fig. 27).

Later, the protuberance is less prominent (fig. 28), and finally it is leveled down to the spherical curve of the body of the united gametes (fig. 29). The

male nucleus with 22 distinct chromosomes proceeds toward the female nucleus, which is in the resting condition (figs. 30, 31), until the male and female nuclei touch (fig. 32). The male nucleus is represented only by 22 crowded chromosomes closely applied to the periphery of the female nucleus (fig. 33); each chromosome of the male nucleus enters into the female nucleus (fig. 34); and finally each chromosome becomes vacuolized and occupies a part of the female nucleus (fig. 34). Later, the fusion nucleus shows no place-distinction of network of both male and female origin.

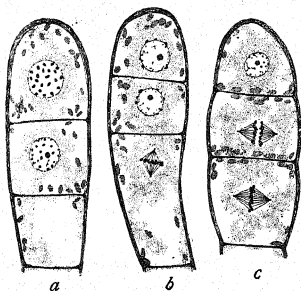


FIG. 12.—Female gametangia in 3-celled stage: *a*, 2 nuclei in prophase, showing 22 chromosomes; *b*, 2 nuclei with chromatin globules around the outside of the membrane; *c*, 2 nuclei in metaphase.

It is a question whether the reticula of male and female origin do occupy distinct places, lying side by side in the fusion nucleus, or whether they intermingle and resume their individuality at the time of chromosome formation. In any event, the fusion nucleus passes into a complete resting condition, with chromatin knots of various sizes and shapes, together with delicate, irregular, discontinuous fibrils,

forming a structure in which the male and female constituents cannot be differentiated by staining.

The formation of the cell wall around the protoplast of the zygote is gradual. Neither the union of the male and female cytoplasm, nor the union of the male and female nuclei seems to be necessary to the formation of the wall, because there is great variability in the interval between the formation of the wall and the process of protoplasmic union, and, moreover, in the cases of zoospores and unfertilized female gametes, the wall is perfectly formed.

The first segmentation division of the sporeling from the fertilized gametes takes place about 24 hours after the union of the

gametes. In early prophase, the nucleus shows 44 chromosomes, all alike both in size and in shape (fig. 35). In middle prophase the chromosomes become more compact (figs. 36, 37). During the formation of the spindle, the nuclear membrane disappears and the equatorial plate is established (figs. 38, 39). The polar view of the plate shows 44 chromosomes (figs. 40, 41). Each chromosome splits longitudinally and half of each proceeds to each pole (figs. 42, 43). The growth of the sporelings holds no strict relation to the mitosis within; the mitosis may take place before the sporeling begins to elongate (figs. 17, 39, 43), or more often the sporeling elongates while the nucleus is in the resting condition (figs. 17, 34). The axis of the first division, as a rule, is perpendicular to the growing axis of the sporeling. After telophase the sporeling is divided into two cells (fig. 44). The nuclei in the two cells divide either simultaneously (fig. 49) or one after the other (figs. 45-48). The number of chromosomes appearing at prophase (fig. 44) and metaphase (fig. 45) is 44. After the 3-celled stage, the growth of the sporeling is not very uniform. The numerous cases observed showed great variability in the method of developing into the multicellular condition. But one principal fact that holds true in almost all cases is that when the sporeling has reached the 2 or 3-celled stage, the cell divisions occur chiefly in a single terminal cell or in two upper cells, while the basal cell

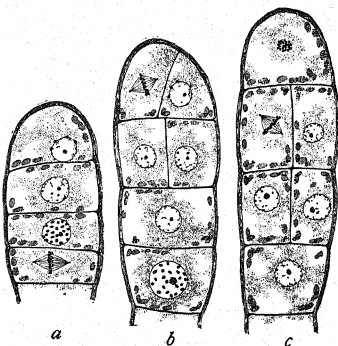


FIG. 13.—Female gametangia with 4 tiers of cells: *a* and *b*, a nucleus in each with chromatin accumulations around the outside of the membrane and a nucleus in each in prophase, showing 22 chromosomes; *c*, nucleus in anaphase viewed from the pole, showing 22 chromosomes.

never divides further. The basal cell is an elongated portion of the sporeling, predetermined in its unicellular stage. Some of the sporelings up to the 7-celled stage are shown in fig. 17.

The sporelings in the cultures, after the 10 or more-celled stage, continue to develop in one direction. The cultures were watched and examined carefully every 5 days up to 30 days, when the plant was still in the filamentous condition (fig. 18, *a*). The filament with a holdfast at the base is unicellular, except near the base,

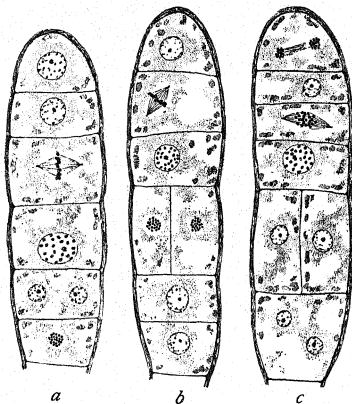


FIG. 14.—Female gametangia in 6 tiers of cells: *a*, *b*, *c*, a nucleus in each in prophase and some in anaphase viewed from the pole, showing 22 chromosomes.

where it is often multicellular. The filament is strikingly like the early stage in the sporelings of *Aglaozonia*. Later, this primary filament does not continue in the upward direction, but produces laterally at its base a number of filaments one after another and side by side, which fuse so as to form a funnel or cup, expanded upward and narrowly constricted downward. Upon the expanded upper margin of this shallow cup, the terminal parts of these filaments remain as hairs. The structure thus produced in the cultures

presents a striking likeness to the young plant of *Zanardinia* in nature, as it occurs thickly on the rock or broken wooden blocks in sea water.

#### Germination of the unfertilized female gamete

As previously stated, the female gametes after their discharge from the gametangia may come to rest very shortly or swim for as long a time as one hour. At the end of the movement, the female gamete becomes spherical. If the female gametes have failed to be caught by the motile male gametes, they remain as motionless,

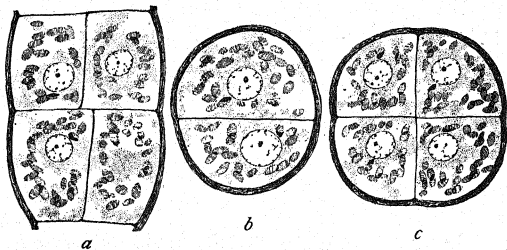


FIG. 15.—Portions of mature female gametangia: *a*, longitudinal section; *b* and *c*, cross sections.

spherical bodies for a considerable time. If they have been fertilized, their nuclei may divide within 24 hours, but when fertilization has not occurred, mitosis is delayed. Even 30 hours after the quiescence, no wall has been recognized (fig. 50). About 46 hours after quiescence a thin wall is developed, and when about 48 hours old, nuclear division begins (figs. 52-57). Every phase in this division is typical and the number of chromosomes is clearly 22 (figs. 52, 55). The elongation of the sporeling begins about 46 hours after quiescence. At the 2-celled stage, one of the two cells which has a thickened wall at its free surface divides once or not any farther, and the cell corresponds to the elongated portion of the sporeling at the unicellular stage. The other cell, which has

no particular thickening of the wall, divides successively and gives rise to all of the subsequent structures. The general morphology of the sporeling in its subsequent development (fig 19) is like that of the fertilized female gamete. The late development of the sporeling was followed up to the stage consisting of about 100 cells. The sporeling at this stage is a filamentous structure whose outer morphological character is hard to distinguish from the filamentous product of the fertilized gametes. Whether the products of the apogamous sporelings would reach maturity is not yet determined.

#### Mitosis in the vegetative cells of zoospore-producing plants

Zoospore-producing plants of *Zanardinia*, in their external morphology, do not differ from gamete-producing plants except in their reproductive organs. The general morphology of the cells composing the thallus is alike in zoospore-producing and gamete-producing plants. The size of the cells in the superficial layers, where the reproductive organs originate, was measured in both plants and was found to be the same.

Vegetative mitosis was studied in the cells of young plants 1.5 cm. in diameter, and in older plants 7 or 8 cm. in diameter. The cells of the hairs upon the margin of the adult plants were also favorable for the study of vegetative mitosis.

The size of the resting nucleus in the superficial cells is about the same as that of the plastids or is even smaller. That the chromatin network is represented chiefly by a number of irregular knots, that the deeply staining globules attached to the outside of the membrane gradually diminish as the quantity of the chromatin knots within increases, that centrosome-like structures are conspicuous only at metaphase and early anaphase, and that the formation of the cell plate at telophase is by means of alveoli of the cytoplasm, are features which repeat almost exactly those described for the gamete-producing plants. The fundamental difference, however, was the appearance of 44 chromosomes, which were counted accurately in prophase and in polar views at metaphase and anaphase.

On account of the cell organization, the zoospore-forming individual of *Zanardinia* cannot be considered as the homologue

of the gamete-producing form, although their similarity might mislead those who observe only the external features.

### Formation of zoosporangia

Zoosporangia are produced on the upper surface of the thallus. In the mature plants, the groups of zoosporangia are distinguished by patches of darker color which contrast sharply with the light-brown color of the sterile portion. These patches are composed of thousands of zoosporangia, produced side by side upon the thallus. The patches look darker because the sporangia, no matter what their age, contain a great number of plastids which have an olive-brown color. The production of zoosporangia begins at a certain spot and proceeds centrifugally, so that the younger

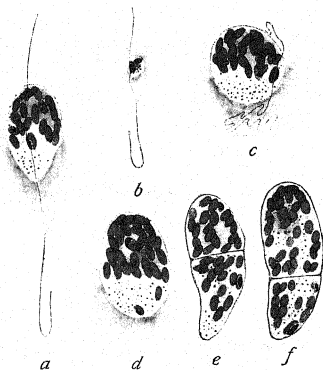


FIG. 16.—Gametes sketched from living material: *a*, female gamete; *b*, male gamete; *c*, female gamete which has assumed the spherical form; *d*, fertilized female gamete; *e* and *f*, 2-celled sporelings from fertilized gamete; in the sporelings large pigment spots are derived from the female gamete and small ones from the male gamete.

stages are generally found at the edge of the patches. The details of the origin of the zoosporangium are as follows: A superficial cell of the thallus begins to swell, elongates slightly and divides, giving rise to two cells, the upper one a zoosporangium initial or zoospore mother cell and the lower one a stalk cell (fig. 20, *a*). The process occurs simultaneously or successively in a number of neighboring superficial cells, so that finally zoospore mother cells are produced in great numbers, crowded closely together. When the superficial

cell divides 1-3 times more, thus producing 3-5 layers of superficial cells, the uppermost cell becomes the zoospore mother cell (fig. 20, b, c). Frequently several cell divisions take place in the superficial cell, so that a superficial cell develops into a filament consisting of more than 7 cells, the terminal one of which becomes the mother cell (fig. 21, a). Moreover, one of the superficial cells, unlike its neighboring cells, may develop into a long multicellular

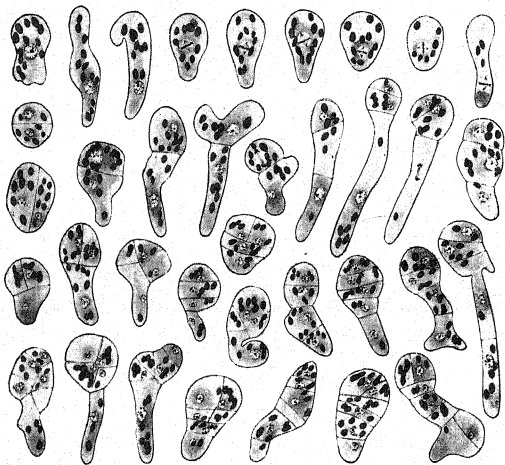


FIG. 17.—Sporelings from fertilized gametes: development in length is dominating.

sterile filament (fig. 21, b), thus indicating the hair origin of the zoosporangia of *Zanardinia*.

The nuclear division of the superficial cell which gives rise to the mother cell and stalk cell is typical, and 44 chromosomes are present (fig. 75). The zoospore mother cell or zoosporangium from a very early stage is distinguished from the sterile superficial cells by being longer than wide (fig. 76). The length of the mother



cell increases as it grows, until it reaches three times its width; then the nuclear changes begin. By this time the mother cell assumes its characteristic ellipsoidal shape, slightly swollen at the top. The nucleus grows with the growth of the cell (fig. 77).

The nucleus in the resting condition contains delicate chromatin fibrils and a nucleolus. Outside the membrane and tightly applied to it there are deeply staining globules (fig. 77). As the chromatin fibrils inside the nucleus grow in quantity, the number and amount of the deeply staining globules diminish (fig. 78). Finally there appear chromatin threads stronger and more continuous than before, and the globules lying outside the membrane disappear completely. It is possible that these globules consist of material closely allied to chromatin, and that they pass into the nucleus, thus contributing to the formation of chromosomes (fig. 79).

The chromatin threads, which are continuous for a considerable distance, run irregularly through the cavity and become more uniform in thickness (fig. 80). They gradually become arranged near the membrane, their parts running parallel by repeated bending (fig. 81), and finally there are established a number of loops of different sizes centering at one part of the membrane (fig. 82). These loops shorten and thicken (figs. 83-85). The loops now show double arms lying side by side; each arm of the loop is a single structure, its origin having been traced from the first indication of a thread structure direct from the chromatin fibrils. The transverse section of these parallel grouped loops is shown in fig. 86; the cut ends of two arms of one loop lie closer than the cut ends of the arms of another loop, and the number of the ends is about 88. The shortening and thickening of the loops proceed, and then they are gradually detached from the main group and form paired chro-

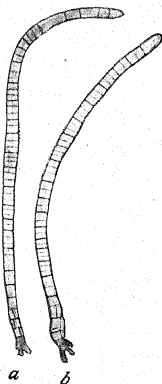


FIG. 18.—Sporelings: *a*, from a fertilized gamete 30 days after fertilization; *b*, from a zoospore 25 days after becoming quiescent.

mosomes, the two arms of each loop forming a bivalent chromosome (figs. 87, 88). After becoming completely detached from the membrane, 22 bivalent chromosomes are scattered throughout the cavity.

The synaptic phase (figs. 79-87) illustrates the mode of origin of the bivalent chromosomes. The chromatin network of the resting stage, irregularly branched and thickened, becomes transformed into the chromatin fibrils in very early prophase, which grow more and more evenly thickened and continuous for considerable distances (fig. 79). This stage would show a double nature, if any such association of two individual fibrils occurs as a premature indication of later *parasyndèse* in the formation of chromosomes, like that described for *Lilium* (GRÉGOIRE 10; BERGHS 3; ALLEN 2), *Polysiphonia* (YAMANOUCHI 19), and in



FIG. 19.—Apogamous sporelings two or three days old.

many other forms. But in *Zanardinia* the fibrils are single (fig. 79). These fibrils gradually become transformed into chromatin threads (figs. 80, 81) which directly form the loops by repeated folding (figs. 81-85). Each of these loops produces a bivalent chromosome, each element of a bivalent chromosome being derived from one of the two bent arms of a single loop. A loop in the synapsis stage, therefore, should be considered as composed of two sporophytic chromosomes associated end to end; the situation is exactly as in *Lilium* (FARMER and MOORE 8), *Fucus* (YAMANOUCHI 20), *Oenothera* (GATES 9), and *Culleria* (YAMANOUCHI 22).

These 22 bivalent chromosomes gather near the center (fig. 80) and then are arranged at the equatorial plate (figs. 91-93). The two elements of each bivalent chromosome separate and proceed to

opposite poles (figs. 94-98). The chromosomes at the poles are aggregated together and two new daughter nuclei are formed (fig. 99). The daughter nuclei increase in size and reach a completely resting condition (fig. 100). The second mitosis takes place simultaneously or in succession, with the two nuclei lying in the common cytoplasm of the mother cell (figs. 101-104), showing clearly in the metaphase 22 chromosomes, the reduced number (fig. 102). The 4 nuclei never divide any farther and never grow larger than the nuclei in the superficial vegetative cells.

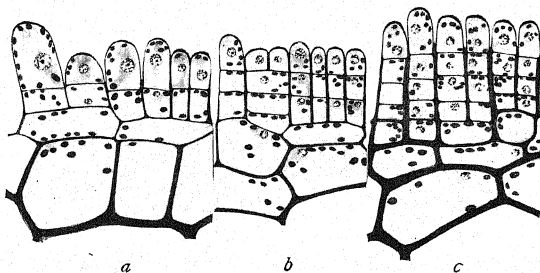


FIG. 20.—Portions of thallus showing the origin of a zoosporangium: *a*, *b*, *c*, 2, 3, and 4 or 5 layers of superficial cells respectively, the outermost of which in each case are rows of zoosporangia.

The relative position of the axes of the two mitoses and the longer axis of the mother cell is variable; the axis of the first mitotic figure is either in the direction of the axis of the mother cell or slightly oblique or at a right angle. In the second division, when the two mitotic figures occur at the same time, the relative position shows all possible directions of the axes. All of these mother cells show no polarity in regard to the axes of the mitotic figures.

When the zoospore mother cell has reached the 4-nucleate stage, cleavage begins at the periphery of the protoplast, proceeds toward the inside, and the protoplasm is quickly cut by curved furrows, that finally divide it into uninucleate masses which are the zoospore primordia (figs. 106-108). The zoospore primordia

round off, leaving a clear space between them and the wall of the mother cell. The nucleus and plastids within a zoospore primordium take a varied arrangement, but there is a cytoplasmic zone entirely devoid of plastids, which becomes the anterior end of the zoospore.

#### The segmentation of the protoplasm in the zoosporangium

When the mother cell has reached the 4-nucleate stage, the position of the four nuclei is either near the central axis or near

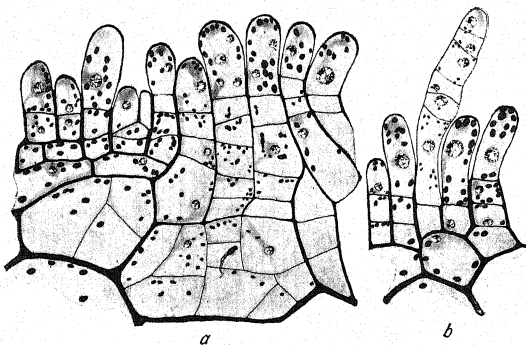


FIG. 21.—Portion of thallus showing the filamentous nature of the zoosporangium: *a*, some of the superficial cells have grown into several-celled filaments, the terminal cells of which have become zoosporangia; *b*, one of the cells equally ranked with neighboring zoosporangia has grown into a many-celled filament, showing the hair nature of the zoosporangium.

the periphery of the cell. Sooner or later the four nuclei become quite uniformly distributed through the protoplasm (fig. 105). Plastids collect in the denser cytoplasm about each of the four nuclei, which become centrally placed (fig. 106). Segmentation takes place by curved cleavage furrows which start at the periphery of the protoplast of the mother cell and cut into the protoplasm (fig. 107). Finally the furrows so divide the protoplasm that it becomes blocked out into four approximately equal, uninucleate

masses which gradually round off as the zoospore primordia (fig. 108).

A zoospore primordium when rounded off contains a centrally placed nucleus with its enveloping cytoplasm bordered by numerous plastids. At this time the primordium shows a nearly radial symmetry, which becomes changed later in connection with the formation of blepharoplasts. The process is as follows: There is first the movement of the nucleus and subsequent displacement of plastids. The nucleus begins to move toward the periphery of the body, displacing the plastids which are in its path. By this movement the nucleus does not quite reach the periphery, but almost all of the plastids are displaced from the region between the periphery and the nucleus, so that this region now contains only colorless cytoplasm. Then in this colorless cytoplasm an indefinite number of deeply staining granules appear, at first 3 or 4, and then more. Similar granules also appear simultaneously around the nucleus and close to it. These granules, with protoplasmic strands between them, are arranged in a row running from the nucleus to the periphery, and the outermost one of these granules lies just inside the outer plasma membrane. Cilia are developed from that part of the outer plasma membrane (*Hautschicht*) just inside of which the outermost one of these granules lies. The outermost one of the granules, the blepharoplast, therefore arises in the cytoplasm, and has a special protoplasmic strand continuous with the nucleus (fig. 22, *a*).

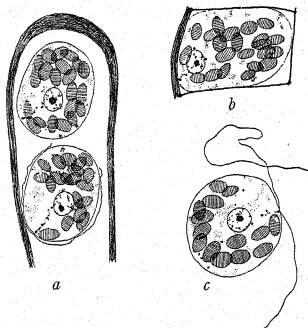


FIG. 22.—Formation of blepharoplast in both zoospore and gamete: *a*, portion of a zoosporangium showing two zoospores, in the lower one of which two cilia are developed from a blepharoplast; *b*, portion of a female gametangium, showing a single cell; *c*, gamete just discharged; red pigment is not differentiated by stains.

The details of the origin of the blepharoplast of the gamete of *Zanardinia* are similar to those of the zoospore (fig. 22, *b*), and both accord with the account already given of gamete and zoospore of *Culleria* (22). For many years there has been considerable divergence of opinion as to the origin and nature of the blepharoplasts of zoospores and gametes of algae. STRASBURGER (16, 17), from an investigation of *Oedogonium*, *Cladophora*, and *Vaucheria*, believed that in these forms the cilia are derived from a body (blepharoplast) arising in the outer plasma membrane, and MOTTIER (12) gave a similar description for *Chara*. DANGEARD (5),

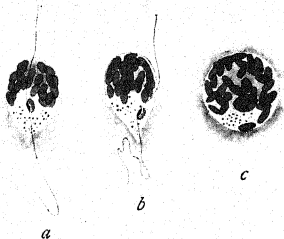


FIG. 23.—Zoospores sketched from living material: *a*, in free swimming condition; *b*, when caught among filamentous algae; *c*, in quiescent condition.

studying *Polytoma*, described a blepharoplast lying at the extremity of the cell directly under the outer plasma membrane, and found a threadlike structure ("rhizoplast") extending from the blepharoplast into the cytoplasm and sometimes ending at the side of the nucleus in a granule ("condyle").

TIMBERLAKE (18) noted in the zoospore of *Hydro-*

*dictyon* that the blepharoplast is a body distinct from the plasma membrane and connected with the nucleus by delicate fibers, but he did not trace its origin. DAVIS (6) traced the origin of the blepharoplast in *Derbesia*. The granules that enter into its composition come from the surface of the nucleus and travel along a system of protoplasmic strands to the plasma membrane beneath which the blepharoplast is formed. Of course, there are numerous descriptions of the blepharoplasts of bryophytes, pteridophytes, and gymnosperms which have been concerned chiefly with its possible relation to the centrosome, but a discussion of these cases is not essential to the present consideration of the origin of the blepharoplast of *Zanardinia*, which has no genetic relations to

such blepharoplasts or centrosomes. That the granule which is the blepharoplast primordium arises in the cytoplasm, and afterward becomes established as the blepharoplast, shows physiological connection with the nucleus holds true in *Polytoma*, *Hydrodictyon*, *Derbesia*, *Culleria*, and *Zanardinia*.

The zoospore in the free swimming condition is oval (fig. 23, *a*, *b*) and usually contains more than 30 plastids. A portion of one of the plastids near the blepharoplast, which lies in the plasma membrane, produces a deep orange color which is the red pigment. The length of the zoospore is  $22.5\ \mu$ ; the cilium directed toward the anterior end is 2 times the length of the zoospore, and the other has the same length as the zoospore.

#### Germination of zoospore

The zoospores were observed to continue in the motile condition at the longest 2 hours and at the shortest only 10 minutes. Toward the end of the movement, the zoospore becomes sluggish, its body gradually assumes the spherical form, and by this time the cilia become tangled and coalescent with the plasma membrane. The formation of the cell wall upon the plasma membrane is gradual. About 4 hours after the zoospores have become quiescent, no wall has yet been formed (fig. 64). In a majority of cases, about 20 hours after quiescence a delicate cell wall is first recognized (fig. 65).

The first segmentation mitosis of the germinating zoospore takes place about 24 hours after quiescence. The nucleus enters prophase at 24 hours, but the metaphase stage is found only in the material fixed 26 hours after quiescence (figs. 67, 68). The number of chromosomes counted at metaphase in polar view is 22, the reduced number (fig. 69). Anaphase and telophase immediately follow metaphase (fig. 70) and the sporeling reaches the 2-celled stage. One of the two cells in the sporeling, which is derived from the elongated portion of the sporeling at the 1-celled stage, either divides once (fig. 72) or remains undivided and becomes the holdfast; while the other cell continues to divide (figs. 71, 73, 74). Some of the sporelings up to the 5-celled stage, obtained in cultures after 2 or 3 days, are shown in fig. 24. In about 25 days the sporeling has developed into a long filament (fig. 18, *b*). Later

at the base of the filament there are produced laterally and in succession a number of filaments whose marginal union forms a funnel or cup, while their free ends appear as hairs growing on the margin of the cup. The outer morphology of this new product of zoosporelings is similar to that of the germinating fertilized gamete.

### Alternation of generations

In *Zanardinia* the plant bearing gametangia has a nucleus with 22 chromosomes in both the vegetative and germ cells, and the number is doubled at fertilization by the union of the sexual nuclei. From this fact, the gamete-bearing plant of *Zanardinia* is the  $x$  generation, and the  $2x$  generation begins at the fertilized

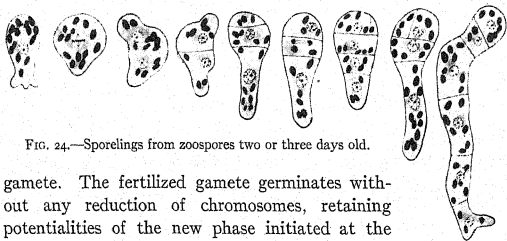


FIG. 24.—Sporelings from zoospores two or three days old.

gamete. The fertilized gamete germinates without any reduction of chromosomes, retaining potentialities of the new phase initiated at the union of the sexual nuclei and having 44 chromosomes. It produces first a filamentous structure from which a cup is developed later with hairs upon the margin. The latter form is similar to the *Zanardinia* plant as it occurs in nature.

On the other hand, the *Zanardinia* plant bearing zoosporangia has a nucleus with 44 chromosomes in the vegetative cells. In the organization of four zoospores from a single mother cell, the number is reduced and the nucleus of the zoospore has the haploid number of chromosomes. From this fact, the zoospore-bearing plant is the  $2x$  generation, which returns to the  $x$  generation during the formation of zoospores. The zoospore germinates with the haploid number of chromosomes and produces a filamentous structure from which there is developed a cup with hairs upon its



margin. The latter form is similar to the *Zanardinia* plant occurring in nature.

In *Zanardinia* plants as they occur in nature, therefore, the  $x$  and  $2x$  generations have similar outer morphological characters. When young, no distinction can be seen, but when the plants become mature, some individuals produce only gametangia, and others only zoosporangia. The cytological study has now shown that the gamete-bearing plant has 22 chromosomes and the zoospore-bearing plant double the number, and also that the product of the gamete-bearing plant establishes the *Zanardinia* plant with 44 chromosomes, identical with the gamete-bearing plants as found in nature. Therefore it is certain that the *Zanardinia* plants in nature which have 44 chromosomes and produce zoosporangia come from the fertilized gametes, and the plants in nature which have 22 chromosomes and produce gametangia come from the zoospores. These two kinds of *Zanardinia* plants are not homologous in character, but fundamentally different from each other, and in the life cycle alternate with each other.

*Zanardinia* plants in the Bay of Naples grow all the year around. The formation of gametes and zoospores is restricted to a certain season of the year. From October to December, the plants are near the adult stage, but few are in reproductive stages, so that from their appearance it is impossible to determine whether they are gametophytes or sporophytes. From early in January to the middle of February, the plants with zoosporangia are abundant, and this season is the climax period of zoospore formation. Toward the end of February and during March, the gametangia-bearing plants are abundant, and this is the season of gamete formation. Then both kinds of adult plants cease to form reproductive organs but may continue to live as perennials with no remnant of reproductive organs. From February to April young sporelings are found and then gradually the larger cups appear. From May till October, the young plants, the product of both gametes and zoospores, grow and attain nearly the adult size in late autumn. It seems evident that zoospores are produced early in the season, in January and February, and that they germinate at once. The production of gametes is a little delayed and the fertilized gametes

germinate during February and March. These two kinds of sporelings grow in the same location, often side by side, during the rest of the season, and from the next October to December the sporelings from zoospores develop into the adult form of the gametophyte and those from gametes into the adult form of the sporophyte. The condition of *Zanardinia* as it occurs in nature is in accord with the cytological evidence.

By this cytological study of *Zanardinia* another type of the brown algae has been shown to have an alternation of generations in its life history.

### Summary

1. The nucleus of the gamete-bearing plants contains 22 chromosomes and the male and female gametes contain the same number.

2. In the union of the gametes the number is doubled, and 44 chromosomes appear in the fertilized sporeling, which develops into the *Zanardinia* plant containing 44 chromosomes.

3. The nucleus of the zoospore-producing plants contains 44 chromosomes, and the number is reduced in zoospore formation, the zoospore containing 22 chromosomes. The zoospore with the reduced number of chromosomes germinates and develops into an individual with 22 chromosomes.

4. It is evident that the gamete-bearing plants come from zoospores and that the zoospore-bearing plants come from fertilized gametes, so that the two generations alternate in the life history.

5. The female gamete of *Zanardinia* may germinate apogamously. There is no irregularity in the mitotic process, 22 chromosomes being invariably present. The individual produced shows external morphological characters similar to those of the product of the fertilized gamete, but the fate of the apogamous individual was not determined.

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#### EXPLANATION OF PLATES I-IV

All figures were drawn with the aid of a camera lucida and Zeiss apochromatic objective 1.5 mm. N.A. 130, in combination with compensating ocular 12; except figs. 2-4, 17, 19-21, and 24, which were drawn with compensating ocular 4; figs. 16 and 23, which were drawn with compensating ocular 8; and fig. 18, which was drawn under Zeiss apochromatic objective 16 mm. combined with compensating ocular 12. The figures are reduced to one-half the original size. Figs. 1-24 are in the text.

#### PLATE I

##### *Union of gametes and germination of fertilized female gamete*

FIG. 25.—Six male gametes which have just stopped the swimming movement: cilia withdrawn, nuclear membrane scarcely visible, and reticulum showing 22 chromosomes.

FIG. 26.—Female gamete which has become quiescent: nucleus in resting stage.

FIG. 27.—Union of male and female gametes: nucleus of male shows 22 chromosomes and that of female in resting condition; no cell membrane has formed around the gametes.

FIG. 28.—Body of male gamete still visible as a slight protuberance on that of female; note individual chromosomes in male gamete nucleus.

FIG. 29.—Cytoplasm of male gamete entirely fused with that of female, and whole body of united gametes about spherical; male nucleus still shows 22 chromosomes.

FIG. 30.—Male nucleus has advanced toward female nucleus.

FIG. 31.—Male nucleus has moved nearer female nucleus.

FIG. 32.—Male nucleus is attached to female nucleus.

FIG. 33.—Male nucleus with 22 chromosomes very closely applied to female nucleus in resting condition: part of cell membrane now thickened.

FIG. 34.—Male nucleus has completely entered into female nucleus: dense chromatin granules are to be seen at part of female nucleus where male nucleus has entered; sporeling elongated at point where cell wall is thickened.

FIG. 35.—Early prophase of fusion nucleus: 44 chromosomes and a nucleolus present; all the chromosomes apparently alike both in form and size.

FIGS. 36, 37.—Prophase: 44 chromosomes clearly shown; fig. 36 a section at right angles to long axis of sporeling similar to that shown in fig. 37.

FIGS. 38, 39.—Metaphase: fig. 38 shows characteristic elongation of sporeling and thickening of elongated portion of cell wall, while in fig. 39 elongation has not yet begun.

FIG. 40.—Metaphase viewed from pole: part of cell wall thickened, but no elongation of sporeling begun.

FIG. 41.—Metaphase: cross section of sporeling perpendicular to axis of elongation; 44 chromosomes plainly visible at equatorial plate.

FIG. 42.—Anaphase.

FIG. 43.—Telophase: although part of cell wall has thickened, no elongation of sporeling has begun.

FIG. 44.—Sporeling in 2-celled stage: nucleus in terminal cell in prophase, and that of basal cell in resting condition.

FIG. 45.—Sporeling in 2-celled stage: nucleus in one cell in metaphase, showing 44 chromosomes; no thickening of cell wall has begun.

FIG. 46.—Sporeling in 2-celled stage: nucleus in terminal cell in late anaphase; basal cell elongated in two directions.

FIG. 47.—Sporeling in 2-celled stage: nucleus in terminal cell in telophase.

FIG. 48.—Sporeling in 2-celled stage: nucleus in basal cell in telophase.

FIG. 49.—Sporeling in 2-celled stage: nucleus in terminal cell in anaphase and that of basal cell in metaphase.

#### PLATE II

##### *Germination of unfertilized female gamete*

FIG. 50.—Female gamete 36 hours after quiescence: cell wall does not seem to be developed except at point where a slight elongation is noticeable.

FIG. 51.—Female gamete 46 hours after quiescence: cell wall now recognizable, especially at elongated point; nucleus in resting condition.

FIG. 52.—Female gamete 48 hours after quiescence: nucleus with 22 chromosomes in prophase.

FIG. 53.—Nucleus in metaphase: contour of sporeling almost spherical.

FIG. 54.—Nucleus in late metaphase, laterally situated: cell wall does not seem to be well formed.

FIG. 55.—Polar view of metaphase showing 22 chromosomes.

FIG. 56.—Anaphase.

FIG. 57.—Telophase: elongation of sporeling is remarkable.

FIG. 58.—Sporeling in 2-celled stage: nucleus in terminal cell in resting condition and that of basal cell in prophase, showing 22 chromosomes.

FIG. 59.—Sporeling in 2-celled stage: nucleus in terminal cell in prophase, showing 22 chromosomes.

FIG. 60.—Sporeling in 2-celled stage: nucleus in terminal cell in metaphase.

FIG. 61.—Sporeling in 2-celled stage: nucleus in terminal cell in anaphase.

FIG. 62, 63.—Sporelings in 3-celled stage: sporeling in fig. 62 has elongated, and that shown in fig. 63 is still about spherical.

*Germination of zoospore*

FIG. 64.—Zoospore 4 hours after quiescence: cell membrane not yet developed; nucleus in resting condition.

FIG. 65.—Zoospore 20 hours after quiescence: cell wall has developed and elongation has begun where wall is thickened.

FIG. 66.—Zoospore 24 hours after quiescence: sporeling has developed in three directions; nucleus in prophase shows 22 chromosomes.

FIG. 67.—Zoospore 26 hours after quiescence: nucleus in metaphase.

FIG. 68.—Metaphase: axis of mitotic figure perpendicular to that shown in previous figure.

FIG. 69.—Cross section of sporeling 26 hours old which still has only a plasma membrane, although its nucleus has advanced to metaphase: chromosomes in equatorial plate at metaphase clearly 22 in number.

FIG. 70.—Anaphase: general contour of sporeling spherical.

FIG. 71.—Sporeling in 2-celled stage: nucleus of terminal cell in prophase, showing 22 chromosomes.

FIG. 72.—Sporeling in 2-celled stage: nucleus of basal cell in early prophase, showing 22 chromosomes.

FIG. 73.—Sporeling in 2-celled stage: nucleus in terminal cell in metaphase.

FIG. 74.—Sporeling in 2-celled stage: nucleus in terminal cell in prophase; comparison of mitotic figures in fig. 73 and fig. 74 shows variability in size.

## PLATE III

*Formation of zoosporangium*

FIG. 75.—One of the superficial cells of a young thallus of a zoospore-forming plant: nucleus in prophase, showing 44 chromosomes; this division will give rise to zoosporangium initial and stalk cell.

FIG. 76.—Portion of superficial cells of a young thallus, showing a superficial cell at the left and a zoosporangium initial and stalk cell at the right.

FIG. 77.—Young zoosporangium initial or zoospore mother cell of characteristic club shape: nucleus in resting condition.

FIG. 78.—Resting nucleus slightly increased in size.

FIG. 79.—Nucleus in very early prophase: chromatin network traversing nuclear cavity.

FIG. 80.—Nucleus in early prophase: slightly more advanced than previous stage.

FIGS. 81-86.—Synapsis.

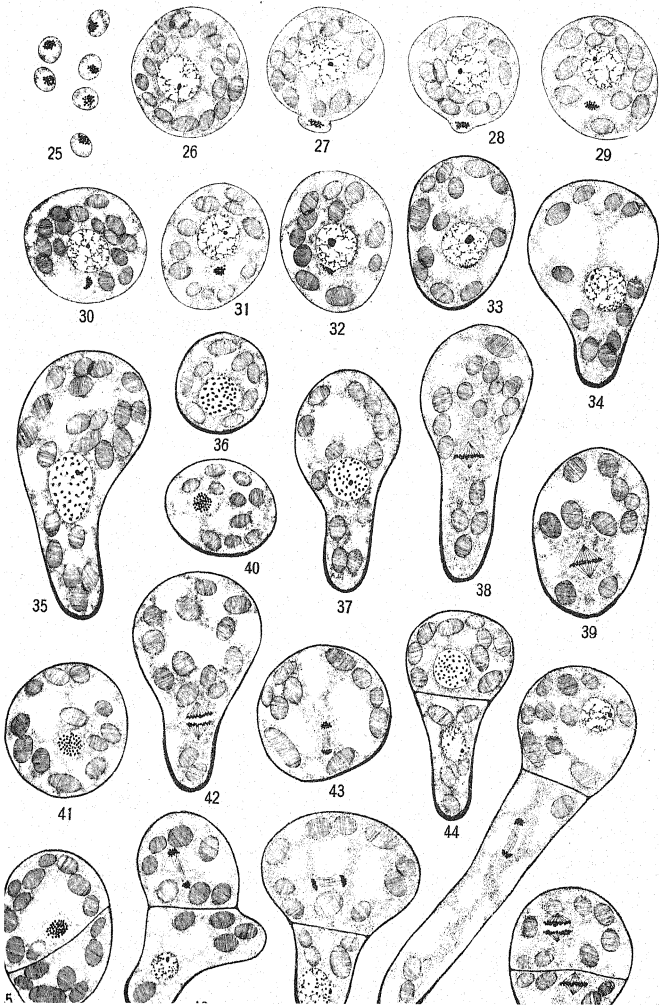
FIG. 81.—Parallel chromatin threads running repeatedly back and forth.

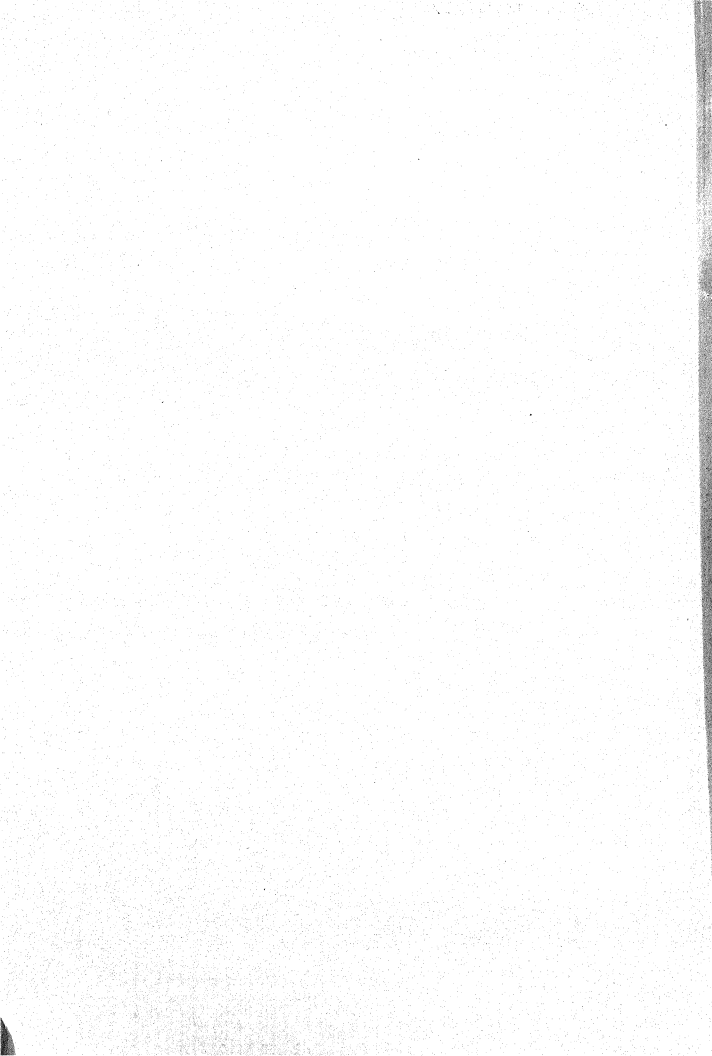
FIG. 82.—Chromatin threads beginning to be arranged in loops which become attached by their ends to nuclear membrane.

FIG. 83.—Formation of loops further advanced.

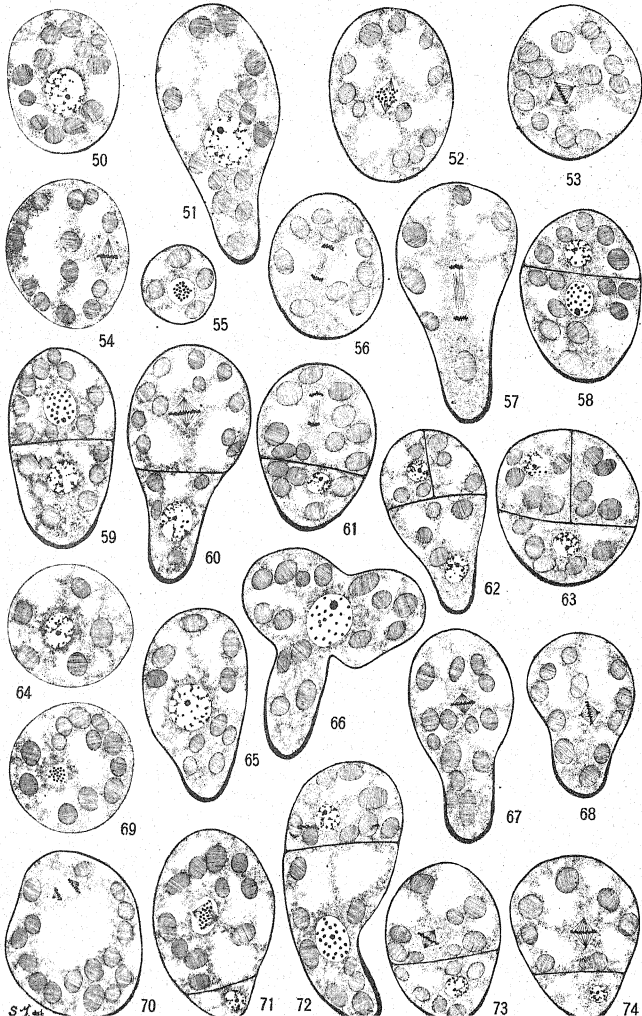
FIG. 84.—Loops shortened and thickened except two belated ones.

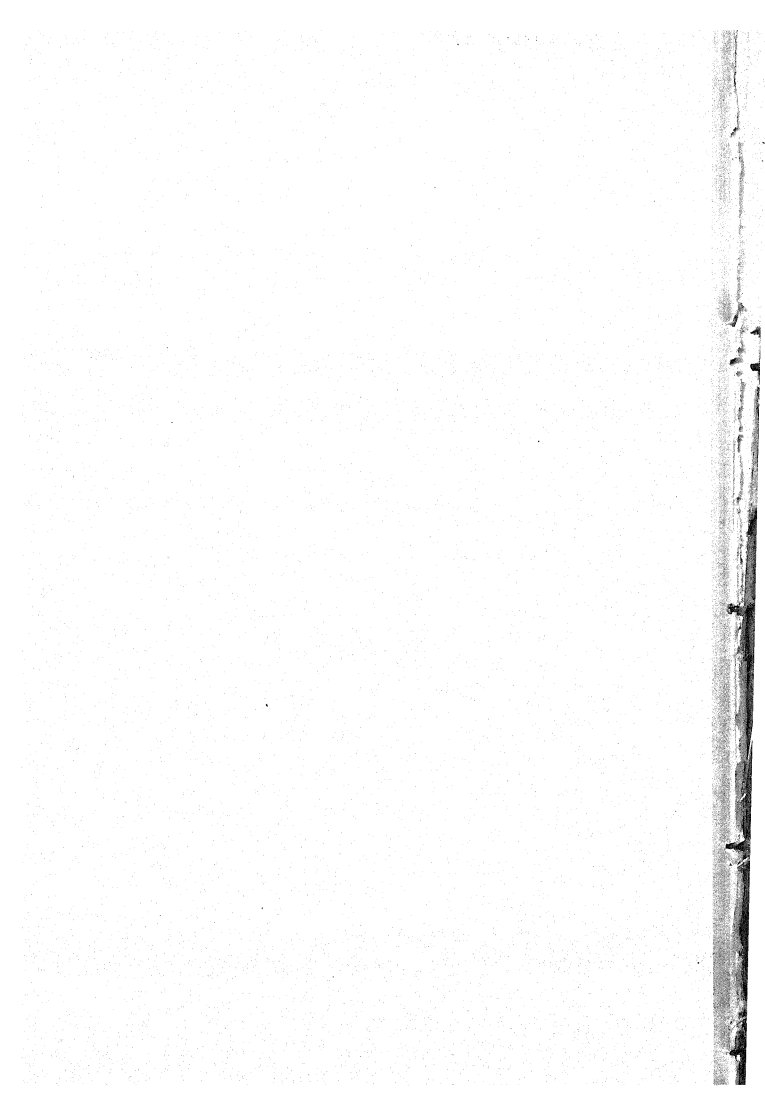
FIG. 85.—Loops shortened and thickened except a single belated one.

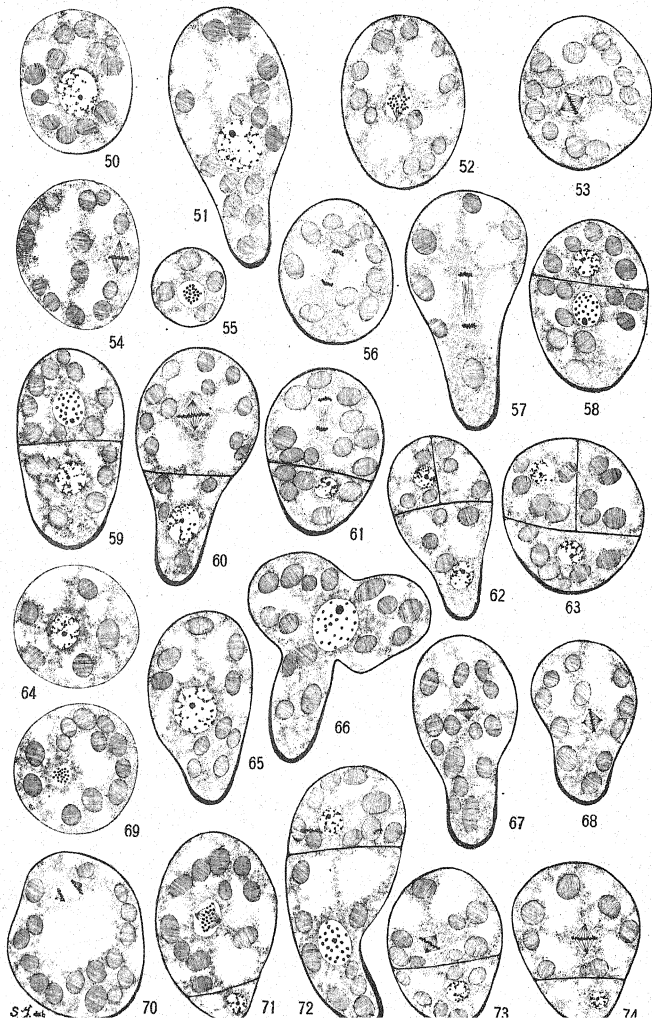




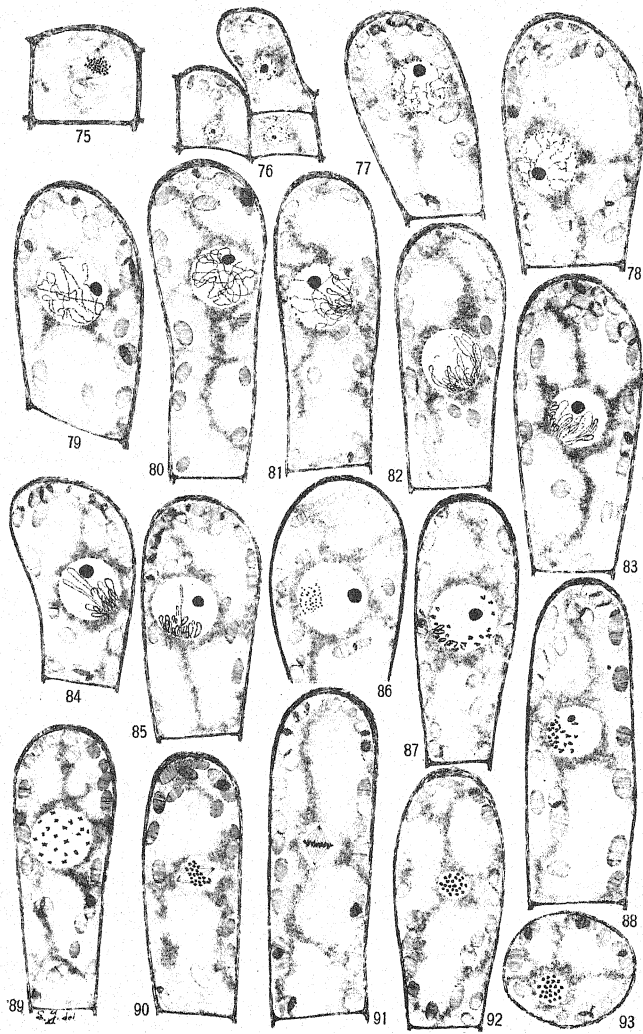


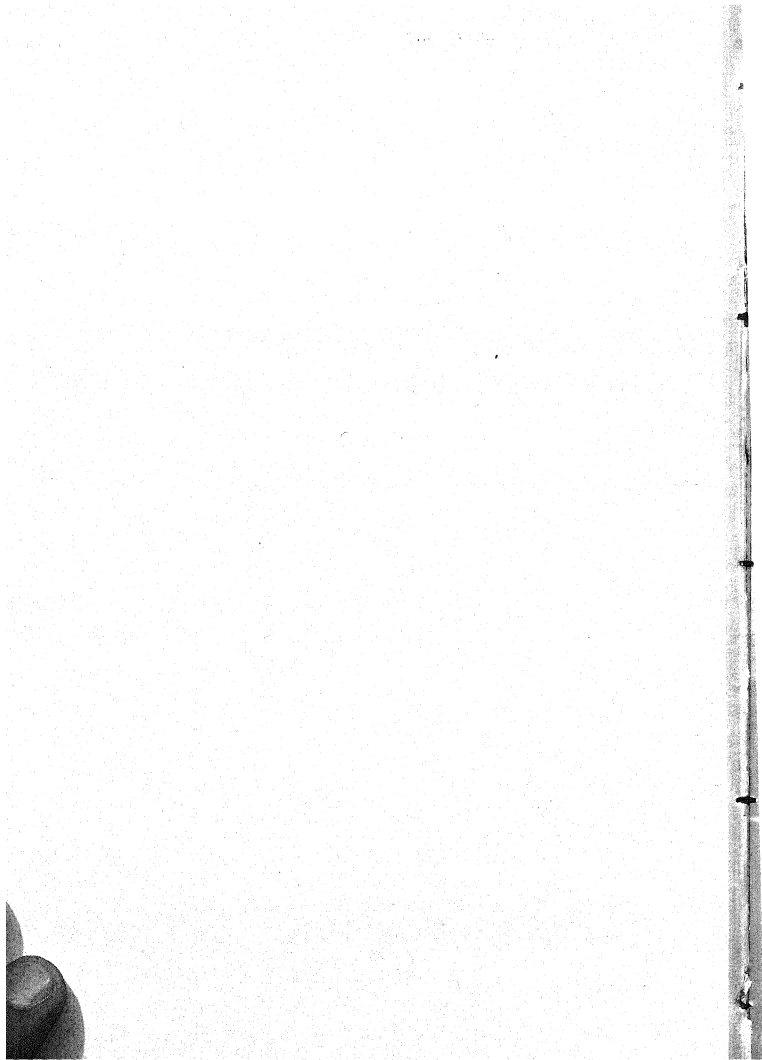












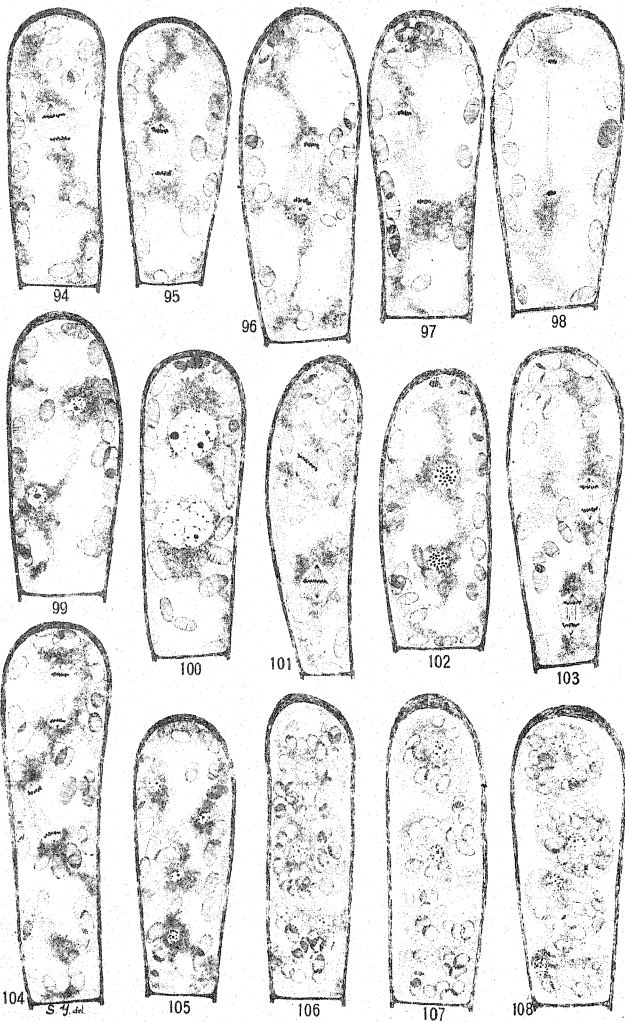






FIG. 86.—Section near base of crowded loops in contact with membrane, showing 44 isolated cut sections of loops.

FIG. 87.—Nucleus emerging from synapsis: several pairs of chromatin loops already in form of chromosomes.

FIG. 88.—Majority of the two arms of each of these loops have developed paired arms of bivalent chromosomes.

FIG. 89.—Diakinesis stage: 22 bivalent chromosomes present.

FIG. 90.—Spindle with two poles just formed.

FIG. 91.—Metaphase, with a centrosome-like body at each pole.

FIG. 92.—Polar view of metaphase, showing 22 bivalent chromosomes at equatorial plate.

FIG. 93.—Polar view of metaphase, showing 22 bivalent chromosomes at equatorial plate; axis of mitotic figure at right angles to that of previous figure.

#### PLATE IV

#### *Formation of zoosporangium (continued)*

As the stage advances, gradual increase of the thickening of the cell wall at the tip of the zoosporangium is noticeable.

FIG. 94.—Late metaphase.

FIG. 95.—Anaphase.

FIG. 96.—Late anaphase.

FIG. 97.—Anaphase further advanced.

FIG. 98.—Telophase.

FIG. 99.—Late telophase: two daughter nuclei in resting condition.

FIG. 100.—Two daughter nuclei have grown quite large.

FIG. 101.—Two nuclei simultaneously in metaphase of second division.

FIG. 102.—Polar view of metaphase, showing 22 chromosomes.

FIG. 103.—Anaphase.

FIG. 104.—Late anaphase.

FIG. 105.—Telophase.

FIG. 106.—Zoosporangium or zoospore mother cell near maturity: plastids moving toward nuclei and surrounding them.

FIG. 107.—Later stage: plastids around nuclei; cytoplasm has become detached from the cell wall and cleavage furrows have appeared; general outlines of individual zoospores established.

FIG. 108.—Mature mother cell containing 4 young zoospores: blepharoplasts and cilia not yet formed.

## THE ORIGIN OF THE ERECT CELLS IN THE PHLOEM OF THE ABIETINEAE

M. A. CHRYSLER

(WITH TWELVE FIGURES)

In the course of a comparative study of the phloem of conifers, certain facts have come to light which seem to demonstrate the origin of the cells found on the margins of medullary rays in their course through the phloem, the so-called "erect cells" of the rays. These cells are distinguished from the other cells of a ray by their elongation in the vertical direction, their almost total lack of starch, and their possession of sieve areas. Of these three criteria, the third is the final one, while the other two afford presumptive evidence of the nature of cells which may be found at the margin of a ray. Such cells are characteristic of the following genera: *Pinus*, *Picea*, *Larix*, *Pseudotsuga*, *Cedrus*, *Tsuga*, *Abies*, and also occur sporadically in *Juniperus* and *Thuja* (7).

In pitting and contents these cells strongly suggest a homology with sieve tubes, as STRASBURGER (5) has pointed out. The same author observed that like sieve tubes these cells lose their contents and collapse as the season advances, while the central cells of a ray enlarge and become laden with starch. In his paper on ray tracheids THOMPSON (7) goes so far as to say that the erect cells are "virtually ray sieve tubes," and, from the fact that marginal cells of both xylem and phloem portions of a ray arise from the same cambial cell, argues that the marginal cells in the xylem are tracheary in origin. The assumption that erect cells are sieve tubes in nature or origin is to be put to a test in the present paper.

There are several possible origins for these cells. They may, like the ordinary (prone) cells of a ray, merge into the pericycle, or they may simply be prone cells which have taken on a new function and acquired a special shape, or they may be cells distinctly added or applied to a ray already existing in a simple condition, that is, constituted only of prone cells.

An obvious method of attaining a solution of the question is

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suggested by the fact that young rays altogether lack erect cells. This may readily be observed in a seedling or in the early layers of growth of a stem or root. Attention has naturally been directed to the stage of growth at which the erect cells make their appearance, and a search has been made among the different organs of the plant for regions where a primitive condition would be likely to appear.

The labors of SCOTT and of JEFFREY have shown that the reproductive axis is one of the places where primitive conditions frequently persist. It has already been shown (3) that ray tracheids are absent from the megasporangiate cone of *Pinus*; it may now be added that a search fails to reveal the presence of erect cells in the phloem region of the cone in *Pinus*, *Picea*, and *Abies*. Accordingly, the reproductive axis yields no results which apply to the present question. The leaf also is not well adapted to the present study, on account of the limited development of the phloem. The seedling, which has been studied with such advantage in other cases, has yielded results of much interest in the present instance, especially when the root is the part used. With the roots of seedlings have been compared young regions of roots of more mature plants.

While *Pinus* has been made the basis of the present study, material of all the genera of Abietineae except *Keteleeria* has been available. Representatives of the hard and soft pines (*P. resinosa* and *P. Strobus*) and of species of *Picea*, *Larix*, *Tsuga*, and *Abies* are abundant around Orono, while material of *Cedrus* and *Pseudolarix* has been kindly supplied by Professor E. C. JEFFREY of Harvard University, and *Pseudotsuga* by Mr. F. D. DAVIS of Missoula, Montana. The pines of this vicinity have been supplemented by *P. rigida* collected by Mr. L. L. WOODS of Wells, Maine, and *P. cembroides*, *P. edulis*, and *P. aristata* through the kindness of Dr. FORREST SHREVE of the Desert Laboratory, Tucson, Arizona. To each of these gentlemen I wish to express my obligation. In providing material the difficulty has been to secure the necessary stages; if the roots are too young the erect cells are absent, if too old the cells have collapsed to such an extent as to obscure their relationships. An age of about four to six years has been found to include the significant stages in *P. Strobus*, but older roots must be

used in the case of nut pines. Whenever possible, the material was preserved in a mixture of corrosive sublimate and picric acid dissolved in 30 per cent alcohol, desilicified in hydrofluoric acid, and imbedded in celloidin. For rendering the sieve areas visible, hematoxylin, according to EHRLICH or especially HAIDENHAIN, proved useful, while a counter stain of safranin was generally employed.

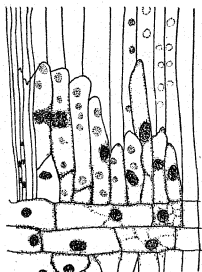


FIG. 1.—*Pinus Strobus*, 6-year root: radial section through phloem in region of a ray, showing long erect cells with sieve pores, and a double nucleus in two cases; the cambial region in this and the other figures lies to the right;  $\times 275$ .

(5, p. 68), is characteristic of young sieve tubes. Immediately to the left of these binucleate cells the crushed and empty older marginal cells are to be seen.

Fig. 2 shows the region bordering on a medullary ray from an 8-year root of *P. cembroides*. On the phloem side of the cambium are to be seen a number of prismatic cells arranged in several series in the radial plane. This layer of cells is intercalated between radial rows of sieve tubes. In some of these cells sieve plates are visible, while the protoplasm becomes more scanty as the older region of the phloem is approached. In other sections such cells may be seen to lose their cytoplasm and nucleus (frequently after

RUSSOW's callus reagent was occasionally of use.

The appearances in *P. Strobus* will be first described. Fig. 1 represents in radial view the phloem region of a ray from the 6-year region of a root of a seedling of this age. The height of the erect cells is here considerably greater than is usually figured, for example, by STRASBURGER (5) and also copied in his *Textbook* (6), and such elongated cells are frequent in young roots. They suggest a close relation of these cells to sieve tubes, as do also the sieve areas which are clearly visible in this section. The cambial region of this and all the other figures illustrating this paper lies to the right. Two of the older erect cells show a doubling of the nucleus, which habit, as STRASBURGER points out

amitosis), and finally become completely collapsed during their second year, after the manner of sieve tubes. On the xylem side a few short tracheids are cut off, but their walls are thin, and they can be traced for only about the width of two tracheids.

Cells in radial groups are frequent in the phloem of young roots in all the species of *Pinus* which have been studied, and their occurrence has been recorded by STRASBURGER (5), who states that they are characteristic of the young regions of the plant, before the tangential rows of phloem parenchyma cells become established. The present investigation goes to show that the radial groups of cells are not only more abundant in the young regions, but that they are more common in young roots than in stems. It should be stated, however, that the cells of the radial rows do not for the most part correspond to phloem parenchyma, as will presently appear. In many cases the cambium for a period of one or more years cuts off no cells on the xylem side, but generally from the median region of such radial groups a narrow medullary ray is developed, as is clearly shown in fig. 3, which is drawn from a 5-year root of *P. resinosa*. Cambial activity dwindles until it is confined to the median region of the radial group, and a medullary ray one cell in height is formed.

It will be noticed that in the xylem region this ray consists of tracheids, though cases occur where the ray cells first formed are tracheids and the later ones are parenchymatous, as shown by the pitting. Thus a tracheidal ray may turn into a parenchymatous one, but the reverse seems to happen seldom if ever. Attention has been called to this point by THOMPSON (7). The initial

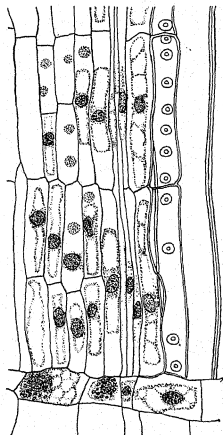


FIG. 2.—*P. cembroides*, 8-year root: radial group of cells provided with sieve pores; at the right the cambium gives rise to a few thin-walled short tracheids;  $\times 275$ .

stages in the development of the xylem portion of such a ray have been observed in numerous instances, and one is represented in fig. 4, from a 7-year root of *P. aristata*. It will be seen that the narrow ray ends on the xylem side in a somewhat pointed cell. This method of formation of a narrow ray differs from the one described by THOMPSON in that the initial activity seems here to be on the phloem side, and the narrow ray is wholly independent of

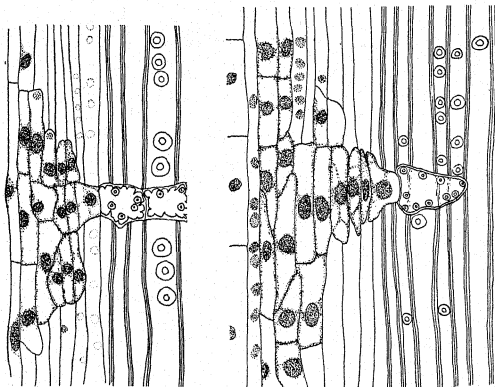


FIG. 3

FIG. 4

FIGS. 3, 4.—Fig. 3, *P. resinosa*, 5-year root: radial plate giving rise to a narrow ray;  $\times 275$ ; fig. 4, *P. aristata*, 7-year root: beginning of the production of a narrow ray;  $\times 275$ .

transitional elements on the xylem side. Instead of giving rise to a single narrow ray, a radial group may be lined up with two or three narrow rays, which, however, consist at first wholly of tracheidal cells. We have here a prolific source of new, that is, secondary rays in the young root. I have observed the formation of parenchymatous rays in the same manner in *Juniperus*.

In fig. 5, from the same root as fig. 3, is seen what frequently takes place when one of the radial groups adjoins a medullary ray.

The cells in question apply themselves to the side of the ray, and as cambial activity in the radial group slackens, this activity is restricted to the border of the ray, thus changing the ray from a simple one consisting only of parenchymatous cells to a ray provided with marginal cells. With fig. 5 may now be compared fig. 1, which shows the same mode of origin for the marginal cells of the phloem region of the ray in *P. Strobus*. Observations have been made on representatives of the hard and soft pines, including the nut and foxtail pines, and appearances similar to fig. 5 have been found in all, when roots of appropriate age were examined.

Regions where two rays lie near one another in the vertical plane present appearances which may readily be explained in the light of what has been said of the radial plates. Fig. 6 shows such a region in a 6-year root of *P. Strobus*. In the upper part of the figure is a ray which on one margin is destitute of erect cells, and on the other (lower) margin is provided with a fringe of much elongated cells which soon merge into a group of prismatic cells forming a radial plate of tissue by means of which the ray shown is connected with another which lies below the area covered by the figure. These cells of the "radial plate" show precisely the same histological features as those previously described, such as the sieve areas which are here shown. At the extreme left of the figure is a row of phloem parenchyma cells, which are easily distinguished from the cells of the radial plate by the starchy contents and swollen shape of the former.

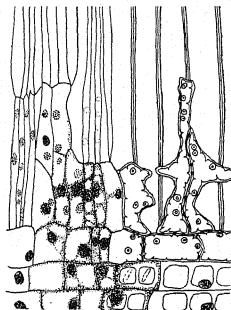


FIG. 5.—*P. resinosa*, 5-year root: a radial plate is in contact with a medullary ray, producing a row of marginal cells;  $\times 275$ .

Further transition stages are shown in fig. 7, from a 5-year root of *P. Strobus*, which represents cases where two rays are closer than those in fig. 6. Almost from the time of their formation from

cambial cells the erect cells of the two rays come into contact, and soon merge into the condition where a single cell spans the space between the rays. As before, such cells lose their contents and become greatly compressed in the radial direction.

Fig. 8, from the same root as fig. 7, represents a case where two rays are practically in contact. A cambial cell is seen to give rise on the phloem side to pairs of cells which appear to have earlier been undivided, as seen in the extreme left of the figure; the nature

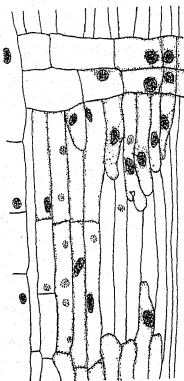


FIG. 6

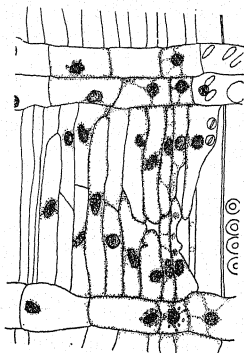


FIG. 7

FIGS. 6, 7.—Fig. 6, *P. Strobus*, 6-year root: region between two rays, with radial plate merging into erect cells;  $\times 275$ ; fig. 7, 5-year root: two rays nearer than in fig. 6.

of these cells is shown by the sieve areas as before. On the xylem side the cambial cell has given rise to irregular shaped tracheids which are evidently of the kind described by THOMPSON (7), and by him regarded as transitional forms between ray tracheids and regular tracheids of the xylem.

It is altogether probable that the prismatic cells lying between two neighboring rays, as shown in figs. 6-8, are of the same nature as the cells of the radial groups, inasmuch as the contents, pitting,



and fate of the cells are the same in both cases. Where two rays are in vertical proximity, it is to be expected that the intervening space will be occupied by one or two cells, but here, as in the case of the radial plates of cells, there is seen a tendency for the cambial activity to be localized in the region of a ray.

Several of the figures illustrate a point which must be emphasized, namely, a ray uniformly begins its course in the outer phloem as a simple structure consisting exclusively of "prone" cells which at certain times of year contain abundant starch, and

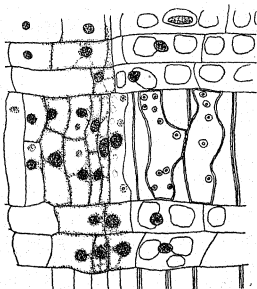


FIG. 8

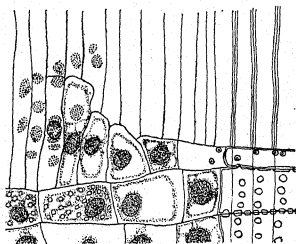


FIG. 9

FIG. 8, 9.—Fig. 8, *P. Strobus*, 5-year root: two rays almost in contact; fig. 9, *P. aristata*, 7-year root: origin of erect cells by cutting off a cell from the end of a sieve tube;  $\times 275$ .

only after cells of a radial group become applied to its margin does the ray come to have a border of "erect" cells. Such a border is usually added to only one edge of the ray at a time, though cases occur in which borders are added to both edges simultaneously.

A slightly different mode of origin of the erect cells has been observed in a number of cases. One of these is shown in fig. 9, from a 7-year root of *P. aristata*. Beginning at the left it will be seen that the ray is not provided with a border of erect cells, but that sieve tubes come in contact with the ray. A little farther to the right an elongated cell provided with sieve pores is cut off from a

sieve tube, and immediately a border to the ray is produced, with the erect cells as usual coterminous with marginal tracheids. Similar instances have been found in other species of *Pinus*, and the transition from sieve tube to erect cell is not always as abrupt as in the case figured. Since the cells of the radial rows are sieve tubes in all respects save length and the nucleus, the occasional formation of an erect cell by cutting off a segment from a sieve tube does not seem surprising. In fact it does not seem necessary to assume an

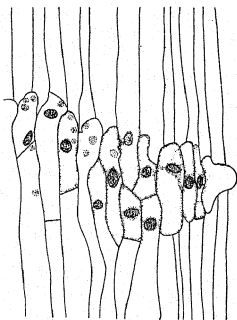


FIG. 10.—*P. Strobus*, 4-year root: sieve tubes have cut off cells from their ends, resulting in production of a ray;  $\times 275$ .

absolutely uniform mode of origin for the erect cells, in view of the fact that a certain cambial cell may be giving rise to ray tracheids, but for some reason suddenly cut off a parenchyma cell instead. Similarly a row of ray cells of the phloem may at first be albuminous, but later-formed members of the row may contain starch. We are interested here, however, in the question as to the evolutionary origin of the erect cells, rather than in occasional modes of origin.

An interesting mode of formation of a ray, showing the tendency of sieve tubes to cut off a cell from the end, has been observed a few times. An example of this mode, from a 4-year root of *P. Strobus*, is represented in fig. 10. In this case the habit of cutting off a cell from the end of a sieve tube has become so well established that a narrow ray has originated, as seen in the cambial region at the right. This process is entirely similar to the mode of origin of erect cells described in the preceding paragraph.

Although the most significant results have been obtained from a study of the young root, the stem of *Pinus* has also been examined to some extent, but the mode of origin of the erect cells in this case was so indefinite compared with what may be seen in similar regions

of the root that the study was soon discontinued. THOMPSON's statement with respect to the ray tracheids may be aptly quoted in this connection (7, p. 108): "The root is admittedly more conservative than the stem. Accordingly in the latter the evolutionary processes are not so well represented. There is a hurrying over of the early stages, so that an actual series can rarely be observed."

Examination of the stem of a number of seedlings of *P. Strobus* and *P. resinosa* showed that (1) the rays in both the xylem and the phloem regions were without marginal cells for several years; (2) the marginal cells as a rule make their appearance slightly earlier in the phloem than in the xylem. This feature is shown clearly in roots as well; in fig. 11, from a 5-year root of *P. resinosa*, is a triangular cambial cell which has cut off a succession of erect cells, while as yet no cells have been cut off on the xylem side. Fig. 7 shows the same feature, and with it may be compared the condition represented in fig. 4. Addition of the marginal cells does not begin in all or many of the rays simultaneously, but after the plant is two to three years old a few of the rays acquire the border.

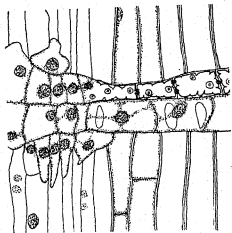


FIG. 11.—*P. resinosa*, 7-year root: the cambial cell on the lower side of the ray cuts off cells on the phloem side but not on the xylem side;  $\times 275$ .

With respect to the remaining genera of Abietineae, only a few species have been studied with any approach to thoroughness, but enough has been done to show that many of the features figured in this paper occur in *Picea*, *Larix*, *Tsuga*, and *Abies*. The same radial groups of cells occur, and the phenomena seen where two rays are vertically contiguous may sometimes be seen. For instance, fig. 6 might be almost duplicated from a young root of *Tsuga canadensis*.

The genus *Abies* presents points of interest, on account of the fact that most species lack marginal cells in the xylem, though they are generally present in the phloem. THOMPSON points out that the erect cells are "never in line with the parenchyma cells of the ray,

but always above or below them. Often they are in line with two or three degenerating cells on the wood side" (7, p. 112). The conclusion seems justified that *Abies* represents a genus which has descended from ancestors which possessed ray tracheids, and is less primitive than *Pinus*. In spite of this, it may sometimes be made out in young roots of *Abies balsamea* that the erect cells have the same mode of origin as has been described for *Pinus*. Fig. 12 illustrates this point, as well as the fact that the triangular cambial cell gives rise to erect cells but not to ray tracheids. In many

instances the shadowy remains of marginal tracheids appear in these root sections.

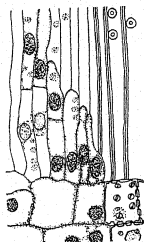


FIG. 12.—*Abies balsamea*, 8-year root: the erect cells have the same mode of origin as in *Pinus*; the cambial cell at margin of the ray cuts off erect cells but no ray tracheids;  $\times 275$ .

With this persistence of marginal cells in the phloem may be compared their earlier appearance in the phloem than in the xylem, as seen in seedlings of *Pinus*. Just why a cambial cell should be more apt to cut off segments on the phloem side than on the xylem side is hard to say; since the supply of food is on the phloem side, unequal nutrition may be the cause at work. But it is possible that we must consider the phloem to be a more conservative region than the xylem, in which case the observation has interest from the phylogenetic standpoint. The latter view of the case is supported by my observations on *Juniperus*, in which genus PENHALLOW (4)

found ray tracheids occurring sporadically. If a radial section of *J. communis* is cut so as to include phloem as well as xylem, it is seen that where ray tracheids occur near the cambium they are coterminous with erect cells. But in the root of *J. virginiana*, in which species ray tracheids have not been reported, a number of instances have been found where the phloem portion of a ray is provided with a border of well marked erect cells, ending at the cambium in a blunt cell, reminding one of the appearances in *Abies balsamea*. Judging by the leaves, *J. communis* is a more primitive species than *J. virginiana*, hence we are prepared to find ancestral features per-

sisting in the former which have been lost in the latter, that is to say, marginal cells occur in the xylem and phloem of *J. communis*, while they are restricted to the phloem of *J. virginiana*. If, as recent studies indicate, the Cupressineae are derived from large-leaved conifers such as the Abietineae, the persistence of marginal cells in the phloem after they have disappeared from the xylem must be interpreted as the retention of an ancestral feature, which amounts to saying that the phloem is a comparatively conservative region.

The genus *Cedrus* shows in a marked way the belated appearance of the ray tracheids compared with the erect cells, but a detailed consideration of *Cedrus* and *Pseudolarix* is reserved for separate treatment in a future paper.

The choice of *Pinus* for detailed study has been made on the basis of its probably primitive nature, in the light of recent paleontological discoveries. If the origin of the erect cells is established for *Pinus*, it holds for the other genera of Abietineae, though they may not so clearly show the formative stages. Within the genus *Pinus* it appears from the work of BAILEY (1) that the nut and foxtail pines are to be regarded as the most primitive, hence *P. edulis*, *P. cembroides*, and *P. aristata* from Arizona were studied with especial interest. Although difficulties were experienced in judging the ages of roots, and seedlings were not available, the material showed the same appearances as had been observed in the eastern pines, as several of the figures indicate. One point was established which forms an additional argument for the primitive nature of these species, namely, the marginal cells in the xylem make their appearance considerably later than in the eastern pines investigated. Since marginal cells are absent from the reproductive axis and from the early rings of growth in stem and root, and particularly because they are not found in the older Pityoxyla (3), they are regarded as of comparatively recent introduction, and it follows that those species in which they appear late in development are to be considered primitive, unless other evidence indicates the contrary. In respect to the time of appearance of the marginal cells, the nut pines and certain of the hard pines, such as *P. rigida*, stand at opposite extremes, according to the limited study which this point has received.

Doubt has been cast by BAILEY (2) on THOMPSON's theory as to the origin of ray tracheids (7), on the ground that the roots used were probably wounded. With this criticism in mind, the material used in the present investigation has been carefully examined for evidences of wounds. Though the possibility of such is not denied, it may be said that the soundest and straightest pieces were selected for study, and no indications of wounds were seen in the sections.

It may be fairly considered that the relation of erect cells to sieve tubes is established, first because of the occurrence in primitive regions of the plant of erect cells so greatly elongated as to be eight times as long as wide, secondly because of their containing much protoplasm but no starch, thirdly because they possess sieve pores, fourthly because they eventually lose their contents and collapse, frequently after the nucleus has divided amitotically. In all of these respects they resemble sieve tubes. To this may be added that they are sometimes cut off from the end of a sieve tube. They are in fact sieve tubes except for possession or rather retention of a nucleus, for the matter of length is a minor one in view of the occurrence in the young root of much elongated erect cells, and the great variation in length of sieve tubes in mature plants of different species, short tubes occurring for instance in the classic case of *Cucurbita*.

In accounting for the origin of erect cells, an examination of admittedly primitive regions of the plant seems to show that they owe their presence on the edge of an originally simple ray to the adhesion, so to speak, of a group of prismatic cells lying in the radial plane. It has been shown that such radial groups are frequent in young parts of the plant, especially the root, and that they occur among radial rows of sieve tubes. But these radial plates of cells represent a passing phase; soon cambial activity in a plate dwindles and becomes restricted to one or a few cells, in which event there are two possibilities: (1) one or more narrow rays may arise, consisting on the phloem side of albuminous cells and on the xylem side of horizontal tracheids, or (2) the cambial activity may become localized at the edge of a ray already existing, producing a series of marginal cells, the so-called erect cells. The earlier members of a

series of these cells are more elongated than the ones formed later, but all possess the same histological features and undergo the same fate. Similar in form and contents are the cells which span the space between two vertically contiguous rays, and which by fission and shortening give rise to a row of erect cells on each of the two rays.

Since the cells of the radial rows occur principally in the young plant, and have the same contents as sieve tubes, with the exception of the nucleus, it is a tempting theory to consider them the fore-runners of sieve tubes, the primary sieve cells which undergo evolution in two directions: (1) lengthening out and losing the nucleus so as to produce sieve tubes, and (2) shortening as they become applied to medullary rays and become converted into erect cells or as they give rise to rays independently. But such a theory must stand on the evidence of a comparative study of phloem of vascular plants, and the evidence is not yet at hand.

### Summary

1. The "erect cells" occurring on the margins of medullary rays in the phloem of most genera of Abietineae do not exist in the young ray, which consists only of ordinary parenchyma.

2. In young roots of *Pinus* the phloem shows certain cells which are essentially short sieve tubes possessing nuclei, occurring in groups in the radial plane. On the xylem side these may merge into one or more narrow rays consisting of tracheids, owing to a diminution and localization of cambial activity.

3. When such a radial group occurs in vertical contact with a medullary ray, cambial activity is sooner or later localized at the edge of the ray, resulting in the production of a border to the ray, such border consisting of sieve cells, which are the erect cells found in mature phloem.

4. Variations of this mode of origin of erect cells occur, such as the cutting of a cell from the end of a sieve tube when it meets the edge of a ray.

5. In young roots and stems marginal cells may make their appearance in the phloem earlier than in the xylem, while in *Abies*

marginal cells have disappeared from the xylem although not from the phloem. This and other observations indicate that phloem is a more conservative region than xylem.

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UNDESCRIBED PLANTS FROM GUATEMALA AND  
OTHER CENTRAL AMERICAN REPUBLICS  
XXXVII<sup>1</sup>

JOHN DONNELL SMITH

**Abutilon Pittieri** Donn. Sm.—Folia orbiculari-ovovata cuspidata basi obtusa integra supra pilis simplicibus aspersa subtus stellato-pubescentia. Pedunculi in axillis supremis singuli graciles. Petala calyce bis et ultra genitalibus bis longiora. Ovarium globosum 10-loculare.

Ramuli lignosi et petioli stipulae pedunculi calyces stellato-pubescentes. Folia nascentia ochraceo-tomentosa, adulta supra glabrescentia 5-7 cm. longa 4-6 cm. lata 7-nervia, nervis ceteris lateralibus utrinque 4-5, venis transversis subparallelis, petiolis 1.5-3 cm. longis, stipulis filiformibus 8 mm. longis caducis. Pedunculi 3-4 cm. longi tenues paulo infra apicem articulati. Calyx hemisphaericus pentagonus 12 mm. longus, lobis deltoideo-ovatis 5 mm. longis apiculatis. Petala in sicco flavescencia basi purpurco-maculata cuneato-ovovata 28-30 mm. longa 21-22 mm. lata biloba, margine basi piloso. Tubus stamineus pubescens cylindricus 7 mm. longus filamenta aequans. Ovarium 5 mm.-diametrale muticum pilosum, loculis 3-ovulatis, stylis stamina aequantibus ad 2 mm. coalitis. Capsula ignota.

El Puente prope vicum *Las Canoas* dictum, Depart. Guatemala, Guatemala, alt. 500 m., Apr. 1905, *H. Pittier* n. 138.—Typus in herbario Musei Nationalis numero proprio 472792 signatus servatur.

**Abutilon pleiopodum** Donn. Sm.—Folia longe petiolata orbiculari-ovata acuta cordata vix ac ne vix lobata integra stellato-pubescentia. Pedunculi fasciculati, axillares racemiflori, terminales uniflori. Calycis partiti segmenta cum petalis dimidio longioribus et genitalibus reflexa. Tubus stamineus conicus. Ovarium hemisphaericum 18-24-loculare.

Ex scheda cll. repertorum arbuscula, tota petalis et genitalibus neglectis stellato-tomentulosa flavicans. Folia supra sparsim subtus dense stellato-pubescentia nervis excurrentibus minutissime mucronulata 7-9-nervia, superiora 8-9. 5 cm. longa 8-8. 5 cm. lata nequaquam lobata, inferiora 19 cm. longa 16. 5 cm. lata levissime triloba, petiolis 4-10. 5 cm. longis, stipulis linearibus 12 mm. longis. Racemi 4-5-ni pedunculo 4-5 cm. longo computato 7-9 cm. longi, bracteis ovatis 3 mm. longis, pedicellis 4-7 mm. longis, floribus confertis.

<sup>1</sup> Continued from BOT. GAZ. 55: 438. 1913.

Pedunculi terminales plurifasciculati 1-2.5 cm. longi. Calyx 6 mm. longus usque ad basin fere partitus, segmentis 4 etiam anthesi peracta coalitis, quinto soluto oblongo-ovato. Petala ex scheda coeruleo-purpurea immaculata fusconervata obovata 9 mm. longa biloba, margine basi barbato. Tubus stamineus pilosus 2 mm. longus, filamentis 4 mm. longis. Ovarium muticum pilosum 3 mm. altum, stylis 4 mm. longis breviter connatis. Capsula deficiens.

Ad fundum *Sepacuite* dictum, Depart. Alta Verapaz, Guatemala, Mart. 1902, O. F. Cook et R. F. Griggs n. 206.—Exemplum typicum in herbario Musei Nationalis sub numero proprio 407992 vidi.

**Comocladia guatemalensis** Donn. Sm.—Foliola 7-juga ovalia vel oblongo-ovalia apice rotundata vel latissime acuta basi retusa integerrima membranacea supra strigillosa subtus uti petiolus et rhachis ochraceo-tomentosa. Fructus maximus dimidio longior quam latior.

Ex scheda cl. repertoris arbusculus ad apicem ramorum versus confertim foliosus. Folia petiolo 4-4.5 cm. longo computato 4.8-5 dm. longa, interstitiis interjugis 3.5-6.5 cm. longis, petiolulis lateralibus vix ullis, terminali 2.5-3.5 cm. longo, foliolis infimis 3.5-4 cm. longis 2.5-3 cm. latis, mediis 7-9.5 cm. longis 5-6 cm. latis, terminali 6 cm. longo 5.5 cm. lato, nervis lateralibus e costa subangulo 60-80° prodeuntibus utrinque 11-14 circiter 5-9 mm. inter se distantibus subtus cum costa patenter et pallide strigillosis, venis transversis haud parallelis. Panicula ochraceo-tomentosa tantum ex fragmentis nota, pedicellis fructiferis solitariis 2 mm. longis. Fructus ellipsoideus 1.5 cm. longus 1 cm. latus, endocarpio cartilagineo, semine 10 mm. longo 7 mm. lato striis nigris ruguloso. Flores non suppetunt.—Foliola ambitu eis *C. Englerianae* Loes. similia differunt tamen integritate indumento etc.

In aridis incultis inter Nentón et Candelaria, Depart. Huehuetenango, Guatemala, Jan. 1906, O. F. Cook n. 59.—Specimina typica duo sub numeris propriis 860255 et 860256 in herbario Musei Nationalis exstant.

**Dalea vulcanicola** Donn. Sm.—Folia cano-sericea, foliolis bijugis oblongo-ellipticis utrinque obtusiusculis carnosis conduplicatis punctatis. Spicae racemosae pedunculatae graciles nutantes cano-villosae, bracteis setaceis. Calyx obliquus, dentibus setaceis. Legumen semiorbiculare indehiscens.

Suffrutex, caulibus strictis virgatis parce breviterque ramosis pubescentibus. Folia 10-11 mm. longa, foliolis 3-5 mm. longis, terminali oblongo-obovato, petiolo 3 mm. longo, rhachi 2 mm. longa, petiolulo terminali 0.5 mm. longo ceteris vix longiore, stipulis setaceis 2-3 mm. longis coloratis pilosis. Spicae 10-20 in racemum terminalem 12-16 cm. longum pubescentem dispositae, vel oppositifoliae et solitariae, 1-2.5 cm. longae pedunculis passim minuteque

bracteatis paulo breviores densiflorae bracteis 2-3 mm. longis basi ipsa semiorbicularibus munitae. Calycis solum fructiferi noti tubus campanulatus 2 mm. longus glaber purpureus parce glandulosus basi callosus, dentes inaequales circiter 1 mm. longi pilosi. Legumen e calyce lateraliter fisso semiprotrusum 1.5 mm. longum 2.5 mm. latum scarosum pallide aureum superne pilosum vix glandulosum, stylo persistente 2.5 mm. longo glabro. Semen loculo conforme unicum altero abortivo comitatum. Petala staminaque deficientia.—Species ob inflorescentiam insignis.

Volcán Atitlán, Depart. Sololá, Guatemala, Febr. 1906, *W. A. Kellerman* n. 5780.—Typum in herbario Musei Nationalis numero proprio 860997 signatum vidi.

**Dioclea** (§ EUDIOCLEA Benth.) **trinervia** Donn. Sm.—Glabra. Foliola ovalia vel oblique ovata cuspidato-acuminata basi rotundata 3-nervia. Calycis lobi subaequales rotundati, vexillum reniformi-cordatum. Carina erostrata. Legumen oblongum sutura superiore rectum inferiore arcuatum, seminibus 1-4 subglobosis.

Frutex volubilis. Foliola 8-10 cm. longa 5-6 cm. lata, nervis lateralibus praeter basales utrinsecus 3, petiolis 6-9 cm. longis, petiolulo terminali 1.5-2.5 cm. longo, stipulis 3 mm. longis et stipellis elongato-triangularibus striatis basi non productis. Racemi duabus partibus floriferi 3-5 dm. longi sulcati puberuli, rhacheos interstitiis internodialibus 1-2 cm. longis, nodis racemoso-floriferis 4-5 mm. longis, pedicellis circiter 6-8-subfasciculatis 5-8 mm. longis apice bibracteolatis, floribus ex scheda Tuerckheimiana sordide violaceis. Calycis tubus 4 mm. longus, lobus superior emarginatus, 3 inferiores semiorbiculares 4 mm. longi atque lati. Vexillum transversim ovale 1 cm. longum 1.5 cm. latum emarginatum supra unguem lineari callosum, sinu basali marginibus inflexo. Alae oblongo-obovatae 14 mm. longae exauriculatae. Carinae petala alis aequilonga angulo recto inflexa obtusa. Antherae uniformes. Stamen vexillare prope basin denticulo semiorbiculari munitum. Ovarium 5-6-ovulatum, stylo superne unifariam barbato. Legumen glabrum turgidum plerumque tetraspermum 8-10 cm. longum 3-3.5 cm. latum sutura superiore vix dilatatum, seminibus atricoloribus 17-19 mm. longis 15-16 mm. latis leviter compressis hilo cano usque ad  $\frac{2}{3}$  fere cinctis, funiculo in appendicem capillaceam hilo aequilongam producto.

GUATEMALA: ad fundum *Sepacuite*, Depart. Alta Verapaz, Mart. 1902, *O. F. Cook* et *R. F. Griggs* n. 140, specimen in herbario Musei Nationalis sub numero proprio 407928 servatum; in fruticetis ad Panzal, Depart. Alta Verapaz, alt. 1000 m., Oct. 1912, *H. von Tuerckheim* n. 3909.—COSTA RICA: in fruticetis ad praedium *Tuts*, Prov. Cartago, alt. 650 m., Nov. 1897, *A. Tondus* n. 11450; Barú ad litora Maris Pacifici, Comarca de Puntarenas, Jan. 1898, *H. Pittier* n. 11958, nomen vulgare *Frijol de Playa*; prope Las Vueltas, Tucurique, Prov. Cartago, alt. 635 m., Apr. 1899, *A. Tondus* n. 12743.

**Phaseolus** (§ **LEPTOSPRON** Benth.) **Tuerckheimii** Donn. Sm.—Foliola rhombeo-ovata acuminata basi rotundata integra pubescentia. Racemi folia subaequantes e basi fere floriferi, pedicellis geminis bractea atque calyce bis fere longioribus flora subdimidio brevioribus. Calycis lobus infimus ceteris longior angustior tubo brevior. Vexillum processu intramarginali utrinque appendiculatum.

Caulis volubilis et petioli racemi calyces pubescentes. Folia juniora supra pilosa subtus sericea, adulta 6.5–9 cm. longa 4.5–7.5 cm. lata, petiolis 4.5–9 cm. longis, petiolulo terminali 18–23 mm. longo, stipulis 4–5 mm. longis et stipellis lanceolatis interdum rubro-maculatis. Racemi sulcati pedunculo 4–8 cm. longo computato 1.5–2.5 dm. longi, nodis floriferis superioribus approximatis, inferioribus remotis, bracteis lineari-lanceolatis 4–5 mm. longis, pedicellis 7–9 mm. longis, floribus in sicco ex lilacino albidis. Calycis tubus 3 mm. longus, labium superius emarginatum, inferioris lobi laterales triangulares, medianus elongato-triangularis 2 mm. longus. Vexillum obovatum 15 mm. longum 11 mm. latum ecallosum exauriculatum intra marginem ad 3 mm. supra unguem appendicula semiovali 2 mm. longa atque lata utrinque munitum. Alae spatulato-obovatae 18 mm. longae 8 mm. latae supra unguem 5 mm. longam intus auriculatae. Carina 15 mm. longa lineari-oblonga 2 mm. lata, rostro bispirali. Stamen vexillare prope basin gibbosum. Ovarium cano-sericeum 2–3-ovulatum, stylo superne unifariam puberulo, stigmate obliquo. Legumen ignotum.

Ad montem inter Tactic et Cobán, Depart. Alta Verapaz, Guatemala, alt. 1800 m., Dec. 1907, *H. von Tuerckheim* (n. II. 1536).—Cuesta de Los Borucas, Comarca de Puntarenas, Costa Rica, Jan. 1897, *H. Pittier* n. 10539.

**Platymiscium pleiostachyum** Donn. Sm.—Foliola 3, lateralibus orbicularibus vel late ovalibus terminali cuspidato-ovato triente brevioribus petiolo terminali bis longioribus. Racemi 2–4-fasciculati, floribus arcte approximatis subsessilibus. Calycis dentes inferiores triangulares. Stamen vexillare liberum. Ovarium stipite longius.

Ex omni parte praeter racemi rhachidem pubescentem glaberrimum. Stipulae crassae ovatae 5 mm. longae. Folia serotina tantum visa, foliolis nitidis, lateralibus 5 cm. longis 4–5 cm. latis utrinque rotundatis, terminali 7.5 cm. longo 6 cm. lato basi rotundato, petiolulis lateralibus 4 mm. longis. Racemi ad nodos annotinos defoliatos plerumque 4-ni vel pseudoterminales itaque 8-ni 4.5–8 cm. longi supra medium dense floriferi, pedicellis vix 1 mm. longis, bracteolis ovalibus 1.5 mm. longis. Flores in ramulis suppetentibus plerumque praecoces. Calycis campanulati tubus 2 mm. longus, dentes

1 mm. longi. Vexillum suborbiculare 9 mm. longum 8 mm. latum. Alae oblique oblongo-ellipticae 8 mm. longae 3.5 mm. latae. Carinae petala alis conformia eis paulo minora. Antherae omnes perfectae, loculis confluentibus. Ovarium stipite dimidio stylo bis longius. Legumen ignotum.—Nomen vulgare *Nambar*.—*P. parviflora* Benth. proximum.

In silvis collinis ad peninsulam Nicoya, Costa Rica, Jan. 1900, *A. Tondus* n. 13539.

**Lonchocarpus** (§ *DENSIFLORI* Benth.) **meistophyllus** Donn. Sm.—Foliola 7–9-juga minuta oblonga apice rotundata vel retusa basi obtuse acuta praeter marginem et subtus costam glabra. Pedicelli plerumque sparsi calicem fere aequantes. Calyx leviter bilabiatus vix denticulatus. Vexillum supra unguem callosum.

Frutex orgyalis e basi ramosus, ramulis glabris, petiolis racemisque pubescentibus. Folia petiolo 4–6 mm. longo computato 3–4 cm. longa, foliolis 9–13 mm. longis 5–7 mm. latis glaucis punctatis, venulis minute reticulatis pellucidis, margine revoluta et subtus costa pilosis, petiolulis 1.5–2 mm. longis pubescentibus. Racemi axillares simplices pedunculo 1–1.5 cm. longo addito 4–5.5 cm. longi densiflori, pedicellis saepe oppositis raro ternis 4 mm. longis, floribus glabris purpureis ut videtur. Calyx e basi acuta campanulatus 5 mm. longus margine puberulus, labio superiore rotundato retuso, inferiore 2–3-denticulato. Petala glabra subaequilonga, alis oblique oblongis, carinis arcuatis, vexillo orbiculari 13 mm.-diametrali ceteris paulo longiore bilobocalloso. Vagina staminalis 1 cm. longa. Ovarium stipite 3 mm. longo instructum lineare 8 mm. longum 6-ovulatum. Legumen desideratur.

In apricis ad Cuesta de Quililhá prope Purulhá, Depart. Baja Verapaz, Guatemala, alt. 1400 m., Apr. 1905, *H. Pittier* n. 141.—Typus numero proprio 472795 signatus in herbario Musei Nationalis adest.

**Derris grandifolia** Donn. Sm.—Foliola 9–11 elliptica vel oblongo-obovata utrinque rotundata vel basi acuta discoloria subtus canotomentosa. Pedicelli pedunculo nodiformi insidentes plurifasciculati vel secus fasciculi rhachin sparsi secundi. Calycis dentes 4 tubi trientem aequantes. Stamen vexillare liberum. Ovarium 7–8-ovulatum.

Arbor 8 m. alta (*Heyde* et *Lux* in scheda), ramulis petiolis racemis calycibus fusco-velutinis. Folia petiolo 6–9 cm. longo computato 3–3.5 dm. longa, rhachi 13–17 cm. longa, foliolis 10–20 cm. longis 5–13 cm. latis costa excurrente mucronulata supra nervis pubescentia et ceterum glabrescentia nervis lateralibus utrinque 12–14 subrectis plerumque simplicibus percutsa transversim crebreque venosa, inferioribus raro suboppositis, petiolulis uti rhachis fusco-velutinis, lateralibus 7–8 mm. longis, terminali 17–20 mm. longo, stipellis

nullis. Racemi tetragoni sulcati 17-25 cm. longi supra medium floriferi, pedunculis crassis 1.5 mm. longis demum 3 mm. longis in rhachin 4 mm. longam incurvam excurrentibus, pedicellis 6-10-fasciculatis vel subfasciculatis 3-5 mm. longis, bracteis caducissimis, bracteolis sub flore binis lineari-oblongis 3 mm. longis. Calycis obliqui tubus 8 mm. longus, lobi 2.5 mm. longi, 3 inferiores subulato-triangulares, superiores in unum integrum connati. Calyx anthesi peracta accrescens usque ad 2 cm. longus 5-dentatus, dente infimo maximo, ceteris subaequalibus. Petala absque eis carinae rubris alba ex scheda Tuerckheimiana, vexillo obovato 15 mm. longo glabro, alis uti carinae petala eis conformia falcata ungue 9 mm. longa addita 18-19 mm. longis. Vagina staminalis latere superiore fissa, stamine vexillari ab initio soluto prope basin geniculato. Ovarium breviter stipitatum lineare fusco-velutinum. Legumen deficit.—*Palo Zope* incolarum.—Species calyce manifestius dentato anormalis.

In declivibus ad Cerro Gordo, Depart. Santa Rosa, Guatemala, alt. 1100 m., Aug. 1892, *Heyde et Lux*, n. 3709 ex Pl. Guat. etc. quas ed. Donn. Sm.—Santa Rosa, Depart. Baja Verapaz, Guatemala, alt. 1600 m., Jul. 1908, *H. von Tuerckheim* (n. II. 2323).

***Diplotripsis macrophyllata*** Donn. Sm.—Foliola lateralia 8 subopposita ovalia utrinque rotundata basi retusa cum terminali obovato supra bullata glabra subtus venis elevatis pilosiuscula. Bractee lanceolato-ovatae pedicello longiores. Bracteolae binae cordato-ovatae calycem totum involucrantes. Calycis lobus infimus elongatus.

Arbor excelsa, coma sphaerica, ramulis serotinis sulcatis sicut folia nascentia paniculae calyces fusco-tomentosis. Folia annotina petiolo 10-11 cm. longo computato 37-45 cm. longa, foliolis cujusque paris 5-10 mm. remotis, infimis 6-7 cm. longis 4-5 cm. latis, superioribus 12.5-16 cm. longis 6-7.5 cm. latis, nervis lateralibus usque ad marginem distinctis utrinque 15-17, venis transversis subparallelis, venulis reticulatis, petiolo et rhachi sulcatis fusco-velutinis, petiolulis lateralibus incrassatis 5 mm. longis fusco-velutinis, terminali 23-27 mm. longo apice incrassato. Paniculae pseudoterminalis prope basin ramuli serotini 2-3 approximatae 17-28 cm. longae, ramis paucis suberectis racemiformibus, pedicellis singulis approximatis 2-6 mm. longis, bractea basin pedicelli amplexante 7-10 mm. longa 4-7 mm. lata obliqua decidua, bracteolis 9-12 mm. longis 8-9 mm. latis breviter stipitatis crispato-undulatis eroso-dentatis persistentibus uti bractea utrinque fusco-velutinis, floribus nutantibus. Calyx in alabastro turbinatus lobis imbricatus, tubo demum late campanulato 6-7 mm. longo crasso, lobis intus velutinis, infimo lanceolato 7-9 mm. longo demisso, superioribus leviter connatis et lateralibus ovatis 4-5 mm. longis. Petala crassiuscula subaequilonga 15-16 mm. longa maculato-venosa saltem in sicco purpurea dorso cano-sericea, carinalia leviter cohaerentia alis conformia oblonga obliqua obtusa latere superiore auriculata, vexillum suborbiculare

inappendiculatum ecallosum. Filamenta glabra 10-12 mm. longa, alterna ceteris parum longiora, antheris ovalibus 0.5 mm. longis. Ovarium sessile cum stylo subaequilongo dense luteo-villosum 5-ovulatum, stylo ad apicem versus glabro et leviter incurvo, stigmate terminali, ovulis lineari-oblongis. Legumen desideratur.—Ab omnibus congeneribus ob bracteas bracteolasque insigniter discrepat.

In silvis ad Las Vueltas, Tucurrique, Prov. Cartago, Costa Rica, alt. 635 m., Jan. 1899, *Adolfo Tonduz* n. 12949.

**Mimosa** (§ **EUMIMOSA** DC.; *Pudicae* Benth.) **teleadactyla** Donn. Sm.—Ramuli setosi, aculeis internodialibus. Folia petiolo et rachidibus aculeata, pinnis trijugis remotis rhachidem subaequantibus petiolo paulo brevioribus, foliolis 8-10-jugis oblongis utrinque rotundatis 3-nerviis, nervo marginali appresse spinuloso. Pedunculi gemini petiolo breviores.

Ramuli videntur reclinati in exemplo suppetente usque ad 1 m. longi teretes 4 mm. crassi, setis densis patulis 4 mm. longis fuscis, aculeis sparsis paucis, infrastipularibus nullis. Stipulae setaceae 7 mm. longae. Petioli 7-9 cm. longi cum rhachi communi et partialibus 6-6.5 cm. longis tetragoni sulcati glabrescentes copiose recurvato-aculeati, aculeis saepe bifidis, rhacheos interstitiis interjugalibus 2.5-4 cm. longis, petiolis partialibus 6-7 mm. longis bistipellatis pilosis, petiolulis 1 mm. longis, foliolis nascentibus totis appresse setosis, adultis glabris glaucis 13-15 mm. longis 5-6 mm. latis basi inaequali obliquis, setis rigidis spiniformibus. Pedunculi axillares, inferiores filiformes 5 cm. longi aculeati setulosi, superiores in ramulo elongato aphylo racemiformi parum evoluti. Capitula globosa, floribus tetrameris. Calyx 0.5 mm. longus ciliato-paleaceus. Corolla 3 mm. longa bracteolis bis longior semilobata, lobis ovatis roseis. Stamina 6 mm. longa, filamentis complanatis. Legumen non visum.—In grege *Pudicarum* pinnis remotis insignis.

Santa Rosa, Depart. Baja Verapaz, Guatemala, Maj. 1904, *O. F. Cook* n. 234.—Exemplum typicum in herbario Musei Nationalis sub numero proprio 860086 exstat.

**Pithecolobium racemiflorum** Donn. Sm.—Inerme glabrum. Pinnae bijugae, paris superioris foliolis trijugis, inferioris bijugis, amplis oblongo-ellipticis tenuiter acuminatis basi acutis. Racemi folia subaequant laxiflori, pedicellis corollam fere aequantibus. Legumen cochleatum.

Arbor 6-7-metralis, ramulis folisque glaberrimis. Petioli 3.5-4.5 cm. longi, interstitio inter pinnas 2-3.5 cm. longo, pinnarum superiorum rhachi 7.5-10 cm. longa, inferiorum 2.5-4 cm. longa, foliolis nitidis pellucidis 6-15 cm. longis 2.5-5.5 cm. latis basi subaequalibus, nervis lateralibus utrinque 7-8 simplicibus, glandulis obsoletis, stipulis stipellisque subulatis 2-3 mm.

longis. Racemi axillares 3-3.5 dm. longi superne puberuli supra medium floriferi, pedicellis singulis plus minus remotis 6 mm. longis, floribus gracilibus 5-meris. Calyx obconico-tubulosus 4 mm. longus denticulatus. Corolla tenuiter tubulosa 6-7 mm. longa triente laciniata, laciniis oblongis obtusis uti pedicellus et calyx strigilloso-puberulis. Stamina in tubum corolla paulo breviora connata. Ovarium sessile. Legumen coriaceum bivalve 15-19 cm. longum 7-13 mm. latum margine sinuosum gyris binis tortuam remote 3-5 spermum, seminibus ovalibus 8 mm. longis, funiculo filiformi spirali 3 mm. longo.—Inflorescentia in genere anormalis.

In silvis collinis ad Las Vueltas, Tucurrique, Comarea de Puntarenas, Costa Rica, alt. 600-750 m., Febr. 1899, A. Tondus n. 13060.

**Aralia sololensis** Donn. Sm.—Folia bipinnata, pinnis quadrjugis et impari ternifoliolatis. Panicula pluries verticillato-ramosa, ramulis ulterioribus dichotomis, floribus 4-5-meris. Discus conicus. Styli in umbone connati, stigmatibus capitellatis.

Specimen unicum suppetens mancum, caule fructibusque deficientibus. Planta humilis ut videtur. Folia glaberrima, rhachi petiolo 3 cm. longo computato 10.5 cm. longa, petiolulis communibus lateralibus 1.5 cm. longis, terminali 3 cm. longo, foliolis tenuiter herbaceis pellucido-venosis acuminatis argute serratis, lateralibus subsessilibus lanceolato-ovatis 5-7.5 cm. longis basi rotundatis, terminali lanceolato-elliptico 6.5-9 cm. longo in petiolulum 8-9 mm. longum angustato. Panicula tantum juvenilis visa glaberrima corymbiformis pedunculo 4 cm. longo addito 11 cm. longa, axibus 3-5-nis, inferioribus 2.5 cm. longis, pedicellis gracilibus 3-7 mm. longis, bracteis oblongis 3-5 mm. longis, bracteolis minutis, floribus glabris plerumque tetrameris. Calycis tubus campanulatus 1 mm. longus, dentes ovati tubo paulo breviores. Petala elliptica 2 mm. longa obtusa. Stamina petalis paulo superata, antheris ovalibus 0.7 mm. longis. Discus 0.8 mm. altus. Styli in columna 0.2 mm. longa connati. Cetera desunt.

Prope Patulul, Depart. Sololá, Guatemala, Febr. 1906, W. A. Kellerman n. 5828.—Typus in herb. Musei Nationalis sub numero proprio 861013 servatur.

**Manettia** (§ LYGISTUM K. Schum.) **stenophylla** Donn. Sm.—Tota praeter corollam glaberrima. Folia subsessilia lanceolato-lineariter acuminata basi acuta. Flores 1-2-ni vel in cymam semel bisve trichotomam dispositi inter minimos pedunculo pedicellisque longiores. Corolla calyce triente longior alte divisa, segmentis intus barbatis.

Suffrutex, ramis teretiusculis striatis tenuibus, internodiis perelongatis. Folia 3-4.5 cm. longa 5-6 mm. lata papyracea subevenia margine revoluta, petiolis 0.5-1 mm. longis, stipulis breviter vaginantibus glandulari-pectinatis medio deltoideis et saepe bidentatis. Pedunculi 2-3 mm. longi, cymis 1-1.5



cm. longis, pedicellis 1-2 mm. longis, bracteolis binis linearibus 0.5-2 mm. longis, floribus 7-8 mm. longis ex scheda cl. repertoris albis. Calycis tubus obovatus 2 mm. longus, lobi 4 supra ovarium leviter connati oblongo-ovati 2 mm. longi apice revoluti, sinubus minutissime uniglanduliferis. Corolla hypocraterimorpha 5-6 mm. longa extus glabra, floris longistyli ad  $\frac{3}{4}$ , floris brevistyli ad  $\frac{2}{3}$ , divisa, tubo apice leviter contracto intus subglabro et exannulato, segmentis oblongo- vel lanceolato-ovatis. Stamina prope incisuras corollae inserta, floris longistyli subsessilia, floris brevistyli 3 mm. longa, antheris linearibus 1 mm. longis. Discus pulvinaris. Stylus corollam aequans vel ea 1 mm. brevior, stigmatibus lamellis lanceolatis 0.5 mm. longis. Capsula papyracea obovata 4.5 mm. longa.

In fruticetis apud Las Vueltas, Tucurrique, Prov. Cartago, Costa Rica, alt. 635 m., Jan. 1899, *Ad. Tondus* n. 12969.

**Rondeletia** (§ *ARACHNOTHRUX* Hook. f.) *calycosa* Donn. Sm.—Folia opposita lanceolato-elliptica tenuiter incurvo-cuspidata infra media attenuata discoloria nervis subtus neglectis glabrescentia. Cymae laxae corymbosae, bracteolis coccineis. Calycis segmenta lineari-lanceolata tubo nimis longiora coccinea 3-5-nervia. Corollae tubus gracillimus segmentis calycinis pluribus longior.

Arbusculus dichotomo-ramosus, ramulis petiolis cymis sordide strigilloso-pilosis. Folia nascentia strigilloso-pilosa, adulta supra leviter arachnoidea vel glabrescentia subtus nervis tantum pilosa 6-9 cm. longa 2-3 cm. lata in cuspidem 14-18 mm. longam attenuata, nervis lateralibus utrinque 6-7, petiolis 4-6 mm. longis, stipulis cuspidato-triangularibus 3-4 mm. longis glabris. Cyma terminalis pedunculo 1.5-2.5 cm. longo computato 5-6 cm. longa foliis reductis bracteata, bracteolis lanceolatis 3-5 mm. longis glabris, pedicellis 2-5 mm. longis, floribus 4-meris. Calycis tubus ellipsoideus paulo supra ovarium productus 3 mm. longus pilosus, segmenta inaequalia 4-7 mm. longa nervis 3-5 percellata. Corollae coccineae tubus 15-17 mm. longus vix 2 mm. latus extus strigilloso-pilosus intus totus glaber, lobi subquadrati 3 mm. longi atque lati crispato-undulati utrinque glabri, os nudum. Antherae faucibus insertae inclusae sessiles lineares 3 mm. longae. Stylus ramis linearibus 2.5 mm. longis additis 9 mm. longis. Capsula ignota.

In silvis ad La Palma, Prov. San José, Costa Rica, alt. 1459 m., Oct. 1898, *Ad. Tondus* n. 11633.

**Ipomoea** (§ *STROPHIPOMOEAE*; *Macrosepalae* Chois.) *sepacui-tensis* Donn. Sm.—Ramis petiolisque glandulari-setosa ceterum glabra. Folia orbiculari-ovata cordata triloba margine integra, loborum lateralium nervo medio supra basin folii orto. Pedunculi 3-flori, pedicellis ternis subclavatis. Sepalum extimum ceteris triente brevius. Capsula ovoidea, seminibus margine villosis.

Rami lignosi teretes, setis glandula insidentibus patentibus purpureis. Folia glabra 12-17 cm. longa atque lata usque ad medium acute lobata sinu rotundata 7-nervia paulo supra basin triplinervia, petiolo 5-7.5 cm. longo parcius setuloso. Pedunculi foliis minoribus fulti erecti robusti teretes purpurascens glabrescentes 8.5-13 cm. longi, pedicellis cum calyce continuis prope basin bracteis binis lanceolato-ovatis 5 mm. longis munitis, fructiferis solum notis 3.5-4.5 cm. longis apice 7 mm. crassis. Sepala subcoriacea glabra ovalia obtusa vix mucruncolata, fructifera interiora 30-32 mm. longa, extimo 2 cm. longo 1.5 cm. lato. Capsula coriaceo-crustacea glabra 26 mm. longa 4-valvata, seminibus orbicularibus 12 mm.-diametralibus praeter marginem flavido-villosum glabris. Cetera desunt.—Ad. *I. setosam* Lindl. proximè accedens.

Ad fundum *Sepacuite* vocatum, Depart. Alta Verapaz, Guatemala, Apr. 1902, O. F. Cook et R. F. Griggs n. 590.—Typus in herbario Musei Nationalis sub numero proprio 408299 exstat.

**Cacabus hondurensis** Donn. Sm.—Hispidus. Folia opposita subsessilia lanceolato-oblonga utrinque acuminata leviter crenata supra lepidota. Pedunculi utrinque axillares supra medium foliaceo-bibracteolati. Calyx triente dentatus. Corolla infundibularis ultra trientem lobata. Stamina 4, staminodio deficiente.

Exemplum unicum visum lignosum virgatum simplex 6.5 dm. longum pilis albidis patentibus hispidum supra medium foliis reductis racemiforme. Folia 9-13.5 cm. longa 2.5-3.5 cm. lata in eodem jugo inaequalia subtus nervis venisque hispidula in petiolum 6-7 mm. longum attenuata. Pedunculi foliis 3-6 cm. longis suffulti 1.5-2 cm. longi hispidi, bracteolis 1-1.5 cm. longis. Calyx campanulatus 2 cm. longus basi leviter retusus pentagonus 10-costatus viridis, dentibus triangularibus 7 mm. longis trinerviis margine uti costae hispidulis. Corolla ex cl. repertore alba, glabra 15-nervia picto-reticulata 3.25 cm. longa supra tubum 3 mm. longum sensim lateque ampliata, lobis rotundatis 13-14 mm. longis atque latis imbricatis. Filamenta supra corollae tubum inserta 6 mm. longa crassa basi vix dilatata, antheris medio affixis ovatis 4 mm. longis apiculatis. Ovarium globosum apiculatum, stylo stigmatibus oblongis 2.5 mm. longis additis 12.5 mm. longo. Bacca nimis immatura calyce fere 3 cm. longo inclusa sessilis 11 mm.-diametralis processu conico 2 mm. longo apiculata bilocularis, placentis bifidis. Semina ignota.—Species inter alia praesertim habitu distinctissima.

Llano de La Puerta prope Copán, Depart. Santa Bárbara, Honduras, alt. 900 m., Jan. 1907, H. Pittier n. 1828.—Typus in herb. Musei Nationalis sub numero proprio 578225 adest.

**Salvia** (§ BRACHYANTHAE Benth.; *Angustifoliae* Benth.) **Kel-lermanii** Donn. Sm.—Frutex totus absque corolla glaber. Folia

ovato-lanceolata basi cuneata callososerulata. Racemi foliis breviores, verticillastris approximatis 4-8-floris, bracteis oblongo-ovatis setaceo-acuminatis. Calyx 3-dentatus, dentibus anticis setaceo-acuminatis. Corolla calyce bis longior, tubo dimidio exserto.

Dichotomo-ramosa. Folia tantum superiora visa 6.5-8 cm. longa 2-2.5 cm. lata acuminata ad costam interdum barbata ceterum glaberrima subtus glandulis minutis aureis dense punctulata, nervis lateralibus utrinque 6-7, petiolis 4-5 mm. longis glandulosis. Racemi pedunculo 6-10 mm. longo addito 2.5-4 cm. longi, verticillastris 3-5 mm. remotis, bracteis 6 mm. longis 3 mm. latis seta 2 mm. longa cuspidatis ante anthesin deciduis, pedicellis 2-3 mm. longis. Calyx tubuloso-campanulatus 10 mm. longus 9-striatus glandulis aureis punctulatus, dentibus triangularibus acuminatis 3 mm. longis. Corolla 21 mm. longa supra calycem ventricosa triente bilabiata praeter galeam coeruleam pube articulata barbatam albida glabra. Discus glandulam ovario bis longiorem exserens. Stylus 22 mm. longus superne lateraliter pubescens, lobo antico 2 mm. longo, postico 1 mm. longo.

Santa Maria, Depart. Quezaltenango, Guatemala, Febr. 1906, W. A. Kellerman n. 5628.—Typus in herbario Musei Nationalis sub numero proprio 578705 asservatur.

**Gaiadendron** (§ ENGAIADENDRON Engl.) *poasense* Donn. Sm.—Folia ovata vel elliptica apice acuta basi rotundata vel acuta. Racemi terminales 2-3-ni vel in axillis superioribus singuli, ramis oppositis bialatis, bractea bracteolisque foliaceis calycem excedentibus, floribus 6-7-meris, lateralibus pedicellatis. Calyx integer.

Frutex terrestris erectus dichotomo-ramosus, ramis teretibus, ramulis angulatis. Folia omnia opposita plerumque ovata 4-5.5 cm. longa 2.5-3.3 cm. lata subtus nigropunctulata et in sicco lutescentia margine revoluta, nervis praeter costam subobsoletis, petiolo 6-7 mm. longo. Racemi 7-9 cm. longi angulati, ramis omnibus oppositis 5-6 mm. longis, internodiis 7-9 mm. longis, bractea florem intermedium sessilem fulciete in ramum decurrente 6-13 mm. longa sicut bracteolae duae 5-6 mm. longae foliis consimili reflexa, floribus in sicco flavescentibus, lateralium pedicellis 2 mm. longis, alabastris obtusis. Calyx 4 mm. longus. Petala linearia 13 mm. longa genitalibus paulo longiora. Stamina usque ad medium petalorum adnata, alterna breviora, antheris oblongo-ellipticis 1.5 mm. longis. Ovarium oblongo-cylindraceum, stylo subulato. Fructus ignotus.—*G. Taguae* G. Don proximum.

Secus marginem lacus superioris in summo monte *Volcán Poás* dicto siti, Prov. Alajuela, Costa Rica, alt. 2600 m.; Jan. 1889, H. Pittier n. 814; Nov. 1896, Ad. Tondus n. 10786.

**Euphorbia** (§ **ANISOPHYLLUM** Roeper) **bryophylla** Donn. Sm.—  
Caules prostrati, ramis ascendentibus nanis ramosis totis densissime  
imbricato-foliosis. Folia subsessilia lineari-lanceolata leviter in-  
aequilatera. Involucra terminalia fasciculata, appendiculis binis  
suborbicularibus ceteras multoties superantibus.

Fruticulus, caulibus tenuibus tetragonis 1 dm. longis, internodiis 4-5 mm.  
longis, ramis approximatis e basi compacto-foliosis 2-3 cm. longis cano-pilosis  
prope basin ramosis, ramulis longe ascendentibus. Folia inferiora opposita,  
superiora alterna, nodis vix ullis invicem fere obtegentia membranacea  
superne denticulata obscure binervia herbaceo-venosa ciliata supra glabra  
sultus pilosa saepius erubescens 4-5 mm. longa 1 mm. lata acuminata basi  
obtusiuscula. Involucra subsessilia 3-7-subcapitulata turbinata 1 mm.  
longa pilosa, glandulis 4 transverse oblongis, appendiculis majoribus 1 mm.-  
diametralibus basi latere altero auriculatis crenulatis sanguineis, ceteris minutis  
arcuatis. Perianthium femininum ovale pilosum, stylis distinctis sanguineis  
breviter bifidis. Capsula seminaque ignota.

Santa Rosa inter Salamá et Purulá, Depart. Baja Verapaz, Guatemala,  
alt. 1700 m., Jun. 1904, *O. F. Cook* n. 225.—Typus in herb. Musei Nationalis  
numero proprio 860325 signatus servatur.

BALTIMORE, MARYLAND

## CONTRIBUTIONS FROM THE ROCKY MOUNTAIN HERBARIUM. XIII

AVEN NELSON

[In this paper are brought together a number of interesting species representing various localities. Among the hundreds of specimens submitted to the Rocky Mountain Herbarium each year for verification or identification, a few real novelties invariably appear. The writer wishes to express his appreciation of the fact that many well known botanists, as well as amateurs, cordially permit him to share with them the pleasure of studying their collections, in whole or in part. The names of those who are represented in this paper occur in the notes on the species described. I want to call attention to the fact that two genera, new to Colorado, are included in the list below.]

***Brodiaea Paysonii*, n. sp.**—The membranes sheathing the corms brown and very fibrous, the inner ones prolonged into scarious sheaths 4–8 cm. long and enclosing the base of the glabrous green scapes and leaves: scapes 15–25 cm. high: leaves narrowly linear, at least a half longer than the scapes, rather lax and spreading: umbel few-flowered, the pedicels 10–15 mm. long: bracts ovate-lanceolate, acute, scarious and somewhat petaloid, green-nerved, shorter than fully developed pedicels: perianth white, shading to faint rose-color, 15–20 mm. long; the oblong obtuse segments a little longer than the tube, delicate in texture with a rather strong purplish-green mid-nerve terminating in a blunt hoodlike tooth at apex: stamens 6, in one row, united to each other and to the perianth segments by a delicate transparent membrane: anthers large, 5–6 mm. long: ovary ovate-oblong, about 6 ovules in each cell: mature seeds black, very large and thin, 7–8 mm. broad, the body of the seed less than half as wide as the black membranous epicarp.

Named in honor of EDWIN E. PAYSON, the collector, an enthusiastic student of the Colorado flora in the Montrose High School, class of 1913. He reports this plant abundant on the dry adobe soils, blossoming so early that "by the last of June there is nothing showing above ground except the scape bearing the large triangular dehiscent capsule." Payson's no. 33, Montrose, May 18, 1912, is the type.

**Eriogonum Visheri**, n. sp.—Bushy branched, glabrous or glabrate annual, 15–25 cm. high: stem usually solitary from the crown, branching from the first node, the 2 or 3 branches each branching di- or trichotomously several times, the ultimate branchlets filiform: leaves rosulate at the base, a whorl of fewer and smaller leaves at the first node; blades mostly broadly oval, rather thick, 15–20 mm. long, on petioles either longer or shorter than the blades; involucre turbinate, in the forks or at the nodes, either sessile or on slender erect pedicels 5–10 mm. or more long: flowers solitary or few in each involucre, usually drooping on short pedicels, very small, yellow; the perianth segments narrowly oblong, 1–1.5 mm. long: achene trigonous, ovoid-acuminate, 2 mm. long.

Singularly like *E. mohavense* Wats. in general aspect, but at once separate by its glabrous leaves. Geographically and environmentally very different. Secured by Professor S. S. VISHNER, of the State University of South Dakota at Meadow, Perkins County, August 3, 1912, no. 536.

**Polygonum pannosum** S. S. Sharp,<sup>1</sup> n. sp.—Slender, glabrous annual, 15–25 cm. high, from a slender, rigid taproot: stem branching irregularly from near the base upward, giving the plant a bushy appearance: leaves few, linear, inclined to be revolute, 1–1.5 cm. long, acute, sessile, becoming bractlike above; sheaths lacerate into numerous threadlike awns, scarious on the margin, but with a brown midrib extending from the base to about one-half their length: flowers numerous in long, slender, spicate racemes, often secund, usually two in the axil of each small, subulate bract, both not appearing at the same time or one remaining rudimentary; pedicels short, slender, but slightly exserted, gradually curved downward until the mature flowers and fruits are deflexed: perianth segments white or sometimes pinkish, with a green midrib and base, 4 mm. long: stamens 8: style short, the branches slightly spreading: achene black, smooth and shining, triquetrous, broadly ovoid, 2–3 mm. long.

Most nearly allied to *P. Douglasii* Greene, but at once seen to be different by the slender zigzag skeletonized stems, the minute linear leaves, and the lacerate sheaths. It was found on sandy slopes in the hills near Sheridan, Wyoming, August 26, 1912, my no. 258.

<sup>1</sup> Mr. SHARP is a student in the University of Wyoming.

**Atriplex Greenei**, n. sp.—Pale greenish moderately scurfy annual, with tapering taproot 1 dm. or more long, branching from the crown: the axes of the main branches excurrent, 1.5–3 dm. long, somewhat tortuous or zigzag, with short slender spreading or ascending branchlets from the numerous approximate nodes: leaves very narrowly linear, the lower only 10–15 mm. long, passing into the subulate bracts above: fructiferous throughout, the fertile flowers 1–several at each node: fruiting bracts very small, about 2 mm. long, closely united, narrowly ovate, the obtuse apex often tipped with a small tooth, the backs usually appendaged at the middle or above with few–several small conical or cylindrical papillae.

This species is most nearly allied to *A. tenuissima* A. Nels., BOT. GAZ. 34:257. 1902, but in that the leaves are only 1–7 mm. long, oblong to ovate or broader, and the fruit subpyramidal and tuberculate below the middle.

I dedicate this species to its collector, the indefatigable phytographer, philosopher, and historian, my friend, Dr. E. L. GREENE, who secured it at Rock Springs, Wyoming, August 9, 1896.

**Arceuthobium Blumeri**, n. sp.—Stems branching dichotomously beginning at the first node and then either regularly or often skipping a node, 4–8 cm. long: fertile branches pale-green, with sharply almost wing-angled internodes; the connate scales forming a shallow cup with two very short broadly triangular points (or these sometimes nearly or quite wanting), the cup about 2 mm. broad: fruit narrowly elliptic or almost fusiform, 3 mm. long and half as broad, on a stipe about 2 mm. long, the body blue-green, the wrinkled conical apex and the stipe pale.—On *Pinus strobus* Engelm.

Specimens on which this species is based were secured in the Huachuca Mountains of Arizona, in October 1910, and were communicated by J. C. BLUMER. Apparently this is the first record of this genus on the above species of five-leaved pines. *A. cyanocarpum* has been reported only on *Pinus flexilis* James. *A. divaricatum* Engelm. seems to use the one and two-leaved pines as host.

**Astragalus macer**, n. sp.—Pale and obscurely appressed puberulent: caudex branched, mostly subterranean: stems slender, sparingly branched, 3–5 dm. high, including the raceme: leaves with linear rachis 3–6 cm. long, on which are borne 3–7 linear alternate

or subopposite pinnae 5-10 mm. long; stipules linear-subulate: flowers not in condition but evidently pale or ochroleucous: calyx tube narrowly campanulate, 5 mm. long, the subulate teeth half as long: pod glabrous, strictly 1-celled, dorsiventrally flattened, somewhat keeled by both sutures, linear-oblong, acute at both ends, 20-30 mm. long, 3-4 mm. broad; stipe from scarcely longer to twice as long as the calyx tube: ovules about 10, the alternate ones apparently aborting; seeds flat, nearly as broad as the pods.

Allied to *A. lonchocarpus* Gray, from which it is at once distinct by its solid (not fistulous), slender stem, its smaller flattened pod on a much shorter stipe. Secured by E. P. WALKER<sup>2</sup> on dry foothills, Paradox Valley, Colorado, June 24, 1912.

*VIOLA SHELTONII biternata*, n. comb.—*V. biternata*, Greene, Pl. Baker. 3:12. 1901; *V. Sheltonii* Rydb., Fl. Col., not Torr.

Mr. Payson's specimens make it evident that a form of the far western *V. Sheltonii* does occur in the Rocky Mountains. While it is not strongly marked, it would seem best to keep it as a geographical variety.

*Chylisma Walkeri*, n. sp.—Very slender annual 1-2 dm. high, including the fruiting raceme which is more than half the plant: stem simple, or sparingly branched above, minutely glandular-puberulent and with some long white scattering spreading hairs: leaves few, approximate just below the peduncle or penduncular branches, entire, ovate to oblong-lanceolate, somewhat shaggy with stiffish white hairs: pedicels filiform, in fruit 10-14 mm. long, spreading at right angles (or somewhat deflexed), and bearing the ascending or erect capsule candelabra-like on the assurgent tip, subtended by a small, ovate, hirsute bract: flowers minute (for this genus), only 2 mm. long, yellow: calyx tube 0.5 mm. long, narrowly oval, petals broadly oval: capsules large for the plant, clavate, as long as the pedicels: seeds large, lance-oblong.

Probably allied to *C. scapoidea* (Nutt.) Small, and to *C. Parryi* (Wats.) Small, but at once separated from both by the glandular puberulence and the small flowers, the latter in every way suggesting one of the small-flowered species of *Sphaerostigma*.

E. P. WALKER's no. 200, Paradox Valley, Colorado, July 1, 1912, is the type and was secured on dry "gyp" hills.

<sup>2</sup> Mr. WALKER collected quite extensively for the University of Wyoming, in the vicinity of his home, Paradox Valley, southwestern Colorado, during the season of 1912.



*Azaleastrum Warrenii*, n. sp.—A low stoutish shrub, with grayish bark, the youngest twigs brown or greenish-brown: leaves in terminal fascicles of 3-5, ovate, oval, or obovate, from broadly rounded at apex to subacute, thin, green on both sides, glabrate (even when young), closely studded on the margin with minute gland-tipped hairs, the blade 1-2 cm. long and tapering to the short margined petiole-like base: the leaf bud scales (stipules?) oblong-lanceolate, hirsute with reddish-brown hairs: flower buds with similar reddish-brown hairs but these very few and scattering on the oval brown deciduous scales, on the short stoutish glandular peduncle, and on the backs of the sepals: flowers lateral on the young twigs, solitary or few at the approximated nodes: sepals narrowly obovate-elliptic, same texture and color as the leaves, 7-10 mm. long, obtuse or obtusish, closely beaded on the margin and back with short gland-tipped hairs: corolla campanulate-rotate, 10-15 mm. long and broad; its lobes suborbicular, about as long as the tube: stamens 10; the cells opening by terminal pores; the stoutish filaments shorter than the corolla, softly pubescent below the middle only: pistil stoutish, obscurely pubescent near the base, enlarging upward to the peltate-capitate stigma, the five lobes of which are encircled by a ringlike border: ovary covered with the gland-tipped hairs characterizing the plant: fruit wanting, but the ovules very numerous on the columellar placenta.

This very interesting *Rhododendron* ally was secured by Mr. EDWARD R. WARREN, of Colorado Springs, so well known for his "Mammals of Colorado," July 14, 1911, in Jackson County, Colorado. The material was exceedingly meager, but the request for full notes brought a very interesting letter, from which the following paragraph is quoted:

"What you say about my no. 16 is very interesting. I found it at my camp on the lower slope of Mt. Zirkel, at the head of navigation for prairie schooners on the 'Ute Pass Trail.' If I remember correctly, it was quite abundant. It was a low plant, perhaps not more than a foot high. It was growing on a slope which was free from growing green timber, with many fallen dead logs, and some standing dead stubs. Soil was gravelly. I made the altitude 9,275 feet, and my altitudes checked very well last summer. There were a few scattering aspens growing about. I may possibly be mistaken as to its being a low plant, I made no special notes, but it was a different growing plant from the *Ceanothus* which grew in the region, and not as conspicuous a shrub. I evidently did not collect much of it, for I have but a single

twig left, and am sending you about half the flowers and leaves from it. If it turns out new and you wish to describe it now, I can send you the rest to be deposited as type specimen. It has quite a woody stem."

Its far northern relative *Rhododendron albiflorum* Hook. was raised to generic rank by Dr. RYDBERG in his *Flora of Montana*, apparently very properly, using the name of the section in which it had been the sole species.

**Gentiana polyantha**, n. sp.—Glabrous annual, 3–6 dm. high, erect, consisting of a main axis and usually a pair of slender erect floriferous branches from each of the several—many nodes: leaves numerous, nearly all subtending cymose flower clusters; lower leaves oblanceolate, nearly sessile by a broadish base, 2–3 cm. long; middle stem leaves sessile, somewhat longer, from oblong to lanceolate; gradually passing into linear-lanceolate ones with broad base: the thyrsiform inflorescence often extending nearly to the base of the plant: calyx with 2 ovate-lanceolate foliaceous sepals as long as the corolla tube, and 3 much smaller somewhat unequal nearly linear lobes: corolla blue or purplish; its tube 10–13 mm. long; its ovate lobes about 5 mm. long, a broad lacerate-setaceous scale fully half as long at the base of each.

In floral characters this is singularly like *G. heterosepala* Engelm., but there the similarity ends. As compared with that, the proposed species is unique in the longer acute leaves, the numerous branches and short internodes, the many flowered thyrses (some plants with more than 100 flowers), and the short pedicels. Secured by ERNEST P. WALKER on Iron Springs Mesa, Colorado, August 21, 1912.

**GENTIANA ANDREWSII dakotica**, n. var.—Leaves smaller and more numerous, narrowly oblong, 4–6 cm. long, tapering gradually to both base and apex, obtusish: corolla smaller, less dilated near the middle: stamens shorter, the scarcely adherent anthers extending but little above the middle of the corolla: corolla truncate at the barely contracted orifice, slightly lacerate-dentate on the margin.

Anyone looking over a series of specimens representing *G. Andrewsii* will be struck at once by the marked difference in aspect between the eastern ones and those from the Dakotas. So marked is this that until the flora and fruit characters are examined *G. Andrewsii* is not suggested by the Dakota plants. The differences, however, do not seem to be fundamental, but may well be designated by a varietal name. The finest example at hand is one secured by Professor TUFTE, of the University of North Dakota, at Devil's Lake, August 15, 1911, no. 195.

**Mertensia refracta**, n. sp.—Obscurely appressed pubescent throughout, the minute hairs without pustulate base: stems tufted, rather stout, more or less branched above, 2-4 dm. high (including the ample inflorescence), rather densely and equably leafy except below where the leaves are smaller or wanting: leaves sessile or nearly so, oblong to ovate, obtuse or subacute, 3-5 cm. long, 8-14 mm. broad: pedicels slender, 6-10 mm. long, early reflexed and most of them ultimately refracted: calyx cleft to the base; its lobes linear, softly hirsute, 2-3 mm. long: corolla blue, 1 cm. long; the campanulate throat and limb as long as the broad tube: stamens inserted in the throat; the filaments scarcely as broad as the anthers: crests inconspicuous: style almost as long as the corolla.

The most striking character of this species is the refracting of the pedicels, much as in certain species of *Lappula*, a character I do not recall as occurring in other species of *Mertensia*. In some species some of the flowers are at length reflexed, and in most of them the inflorescence as a whole is more or less drooping, but in this one the individual pedicels are sharply refracted at their very base.

Secured by A. A. GRIFFIN, Wagon Wheel Gap, Colorado, at 9000 feet, July 28, 1912, no. 139.

**Oreocarya paradoxa**, n. sp.—Matted caespitose perennial; the caudex freely branched, the branches short, crowded, covered with dead leaf-bases: stems short, 8-15 cm. long, more or less curved and ascending from the decumbent base, hirsute-hispid: leaves numerous, spatulate-oblancoolate, 1.5-3 cm. long, including the narrowed petiole-like base, subhispid with more or less appressed white hairs: inflorescence at first subcapitate, becoming a short thyrsoïd spike: calyx cleft to the base, its lobes linear-lanceolate, 4-5 mm. long: corolla tube yellow, slender, nearly three times as long as the sepals; the yellowish or nearly white corolla lobes obovate, fully one-third as long as the tube; the crests very prominent: anthers just below the crests: nutlets ovoid, muriculate, attached by the middle to the ovoid gynobase, the free portion of the style twice as long as the mature nutlets.

The yellow-flowered species of *Oreocarya* are not numerous, and the long-flowered ones are even scarcer. This strongly tufted-matted form with its long clear-yellow tube is distinctive. Secured by E. P. WALKER on dry "gyp" hills, in Paradox Valley, Colorado, no. 91, June 19, 1912.

**Pentstemon Griffinii**, n. sp.—Stems solitary from the crowns of the short slender branches of the subterranean caudex, very slender, glabrous, erect, 2–3 dm. high: basal leaves rosulate on the crowns, relatively few, oblong-ob lanceolate, 1.5–3 cm. long, tapering into very short petioles; stem leaves about 3 pairs, those of the lowest linear-spatulate, the others linear and smaller: flowers few, in an open obscurely glandular-pubescent cymose panicle with subulate bracts: sepals ovate, acute, often purplish, with scarious margins, 5–7 mm. long: corolla bluish-purple, tubular-funnel-form, about 2 cm. long, the lips unequal; lower lip with two longitudinal yellow-bearded crests: sterile filament densely bearded for more than half its length with short soft yellow hair; anthers cells confluent but not explanate.

No very near ally of this is known to the writer. Collected by ALFRED A. GRIFFIN in the Rio Grande Valley, on moist east slopes, at 8200 feet, July 28, 1912, no. 145. Mr. GRIFFIN, who is now at the University of Michigan, is connected with the National Forest Service and was in the field in Colorado in the summer of 1912.

**MACHAERANTHERA PULVERULENTA vacans**, n. var.—Probably a short-lived perennial, greenish, the viscosity extending to the branches and stems: stems suberect and sparingly branched: leaves small, mostly linear.

It seems rather superfluous to add new names to our already long list, but the following numbers from WALKER's collections are so aberrant that one hesitates to refer them to a known species without some explanation: no. 360, Paradox, August 1; no. 537, Coventry, Sept. 2; no. 434 (less typical), San Miguel Canon, August 10, 1912.

**Wyomingia vivax**, n. sp.—The shrubby base (caudex) naked, freely branched, 5–10 cm. high; the season's stems very slender, simple, moderately cinereous, 3–6 dm. high: leaves closely appressed-cinereous, scattering on the crowns of the caudex and on the stems, linear or some of them narrowly spatulate, 2–4 cm. long, becoming smaller upward and passing into the small bracts of the inflorescence: heads few-several, in an open corymb, medium size; involucre hemispheric, 7–10 mm. broad, its bracts softly cinereous, in 3 or 4 unequal rows; rays 15–20, pink; disk flowers numerous, small, with sordid pappus shorter than the corolla; achene sparsely pubescent, shorter than the pappus.

The generic position of this plant is not clear. It is aberrant as a *Wyomingia*, but more so as an *Erigeron* or *Aster*. It was secured by E. P. WALKER on the east slope of the La Sal Mountains, Utah, at about 9000 feet elevation. Dry rocky hills, July 30, 1912, no. 355.

***Taraxacum fasciculatum*, n. sp.**—Root slender, with an enlarged crown bearing few—several oblanceolate or oblong obtusish merely dentate or denticulate subsessile or short-petioled glabrous leaves 4–7 cm. long: scapes in small fascicles of 2—several (usually 3 or 4) from the crown, produced simultaneously and subequal in length, in full flower 12–14 cm. long: heads medium size, the short calyculate bracts few or wanting, none reflexed; involucral bracts, 12–14 mm. long, lanceolate to linear, the outer ones notably corniculate (achenes immature in the specimens at hand).

A remarkably fine native species, seemingly very distinct. A. A. GRIFFIN, no. 111, Wagon Wheel Gap, Blue Park, altitude 11,000 feet, July 21, 1912.

ROCKY MOUNTAIN HERBARIUM  
LARAMIE, WYOMING

## SEED PRODUCTION IN YUCCA GLAUCA

MAX M. ELLIS

In the course of other collecting in the autumn of 1912 some data were gathered concerning the amount of seed produced by *Yucca glauca*. It is well known that this plant depends upon the Yucca or Pronuba moth (*Pronuba yuccasella*)\* for pollination, and that the larvae of the *Pronuba* in turn feed upon some of the developing seeds of the *Yucca*. As a result, *Yucca* produces an excess of seed. It is concerning this excess of seed and the number of seeds eaten that data are submitted. Seed pods of *Yucca* were collected and counts made at Boulder and Wray, Colorado.

### 1. Distribution of seed-producing plants

At Boulder counts were made on three mesas, one north and one south of the city, and one near the base of Flagstaff Mountain. On the south mesa 320 plants were counted and no seed pods found. Many of the plants (about half of them) had flower stalks still standing. Some of these stalks were discolored and worn, showing them to be more than a year old, but the majority of the flower stalks were of this year's growth. Occasionally a group of a few plants with old flower stalks, on which were old empty seed pods, was found.

On the north mesa 142 plants were counted and none with seed pods found. Of this number 80 had this year's flower stalks still standing and 12 had old flower stalks.

Only on the mesa near Flagstaff were seed pods found on this year's stalks. Here 210 plants were counted and 10 found with seed pods. These 10 plants were all included within a square of 200 yards.

At Wray observations were made on several mesas on the north side of the Republican River. No attempt was made to count all of the plants seen, but by comparison with the Boulder mesas at

\**Pronuba*, although generally used, is preoccupied. The name should stand *Tegeticula yuccasella*.

least 700 plants were examined. No seed pods on this year's stalks were found, and conditions were essentially the same as at Boulder. About one-half of the plants had flower stalks standing; some of which were old and some this year's growth. Several areas were noticed in which many of the plants bore old stalks with empty seed pods. Wherever these old empty seed pods were found, it was noticed that all the plants bearing them were in a rather restricted area (about 100 yards square), and that most of the plants within this area bore pods. On the south side of the Republican River a group of yuccas was found with seed pods on this year's stalks; 27 plants, all within a rectangle 100 by 200 yards, bore seed pods. Only the very small plants, of which there were 33 within this area, were without seed pods. On the other hand, although yuccas grew on both the east and the west of this group, no other seed-producing yuccas were found for a distance of 300 yards or more on either side. Table I shows that the seed production in this area was high.

TABLE I

Number of plants	Number of pods per stalk
6.....	1
3.....	2
8.....	3
6.....	4
3.....	5
1.....	7

The data collected show that the seed-producing plants are found in occasional small groups. These occasional areas are to be explained as the result of a very local, annual distribution of the *Pronuba* moth, the pollinating agent of this species. It is evident that a large number of yuccas flower every year, and it is hardly possible that all of these flowers are infertile were they properly pollinated. Allowing 10 blossoms to each stalk, it is seen that the number of blossoms produced annually in even a small area is very large. This points to an enormous waste by the species as a whole, as a result of the restricted pollination by a single species of insect, if the yucca moth is always so locally distributed. So far as could be determined, there were no barriers to a flying insect

between the areas of seed-producing yuccas and those without seed-producing plants, yet the local distribution of the seed-producing plants obtained in all cases.

## 2. Seed production

The seed pods as collected in the field were placed in small paper bags. In the subsequent counting of the seeds of each pod three classes were recognized. Seeds of the first class were termed perfect seeds; these were the uniform, well-filled, shiny black seeds which were uninjured, that is, they were apparently perfect seeds so far as could be determined by inspection. The second class included all the seeds injured by the larvae, and they are listed as seeds eaten. As the *Pronuba* larva grows it eats its way up through the center of the column of flat seeds, which are stacked vertically in the pod like a pile of coins. The seeds which had been eaten were usually ring-shaped, as only the central portion was destroyed. The diameter of the area destroyed increased as the top of the pod was approached, since the larva grew as it ascended the column of seeds. Often seeds taken from the top of the pod were almost completely eaten, while those from the bottom were perforated by holes 1 mm. or so in diameter. As a rule, the injured seeds remained in place in the pod and were usually cemented together by the excrement of the larva. There was some evidence that the seeds continued to fill out even after the larvae perforated them, as the seeds so injured were of about the same thickness as the uninjured seeds. The number of white infertile seeds was also apparently the same in the columns of injured seeds as in the columns of uninjured seeds. In the third group, termed imperfect seeds, were placed the infertile white seeds and the few malformed black seeds. In separating the seeds of this group and of group one there was a chance for error; it was often difficult to decide whether a seed was of one class or the other. Since most of the good seeds, however, were so well formed, this discrepancy is within the probable error. The data for the individual pods is given in table II, and the summarized results in tables III and IV. The pods from each stalk are listed separately.

The average number of seeds produced was about 300 per pod.



These were divided about equally between the perfect black seeds and the infertile white seeds. The malformed seeds were few and were generally at the bottom of the pod. The individual data

TABLE II  
BOULDER PLANTS

Perfect seeds	Imperfect seeds	Seeds eaten	Seeds in pod	Number of larvae	Seeds eaten per larva	Per cent per larva
Plant 1						
133	128	60	321	2	30	9
157	150	0	316	1	0	0
165	153	0	318	1	0	0
Plant 2						
172	41	156	369	12	13	4
118	210	46	374	2	23	6
62	141	0	203	1	0	0
Plant 3						
15	102	139	256	3	46	18
5	132	86	223	3	29	13
Plant 4						
177	187	84	448	3	26	6
245	72	17	334	2	8	2
125	187	19	331	1	19	6
172	150	0	322	1	0	0
Plant 5						
72	104	58	234	3	19	8
151	166	1	218	1	1	0.5
Plant 6						
154	61	101	316	1	101	32
118	130	26	274	1	26	9
Plant 7						
232	52	0	284	1	0	0
Plant 8						
232	96	27	355	2	13	4
Plant 9						
125	105	95	325	5	19	6
Plant 10						
136	158	26	320	2	13	4
127	156	8	291	1	8	3

WRAY PLANTS

Plant 11						
72	91	36	189	1	26	14
63	12	195	270	2	87	32
27	18	169	214	3	50	26
31	27	180	238	9	20	9
76	50	147	273	2	25	9
50	50	64	168	2	25	15
Plant 12						
70	141	41	252	2	20	8
107	109	0	216	1	0	0
54	5	187	291	10	19	7

show a tendency at least for the pods on the same stalk to produce about the same number of seeds, although the number of pods per stalk is apparently not a factor. The highest number of seeds taken from a single pod was 448 from a pod of plant 4, which bore 4 pods. The lowest number, on the other hand (168), was from a pod of plant 11, with 6 pods.

TABLE III

	Seeds in pod	Perfect seeds	Imperfect seeds	Seeds eaten	Actual loss in per cent
Minimum.....	168	5	12	0	0
Maximum.....	448	245	210	187	64
Average.....	280	118	104	58	21

TABLE IV

	Number of larvae	Seeds eaten per larva	Per cent of seeds eaten per larva
Minimum.....	1	0	0
Maximum.....	12	101	32
Average.....	2.66	19	7

### 3. Relation of larvae to seed production

The most interesting point in connection with the seed production of *Yucca* is the presence of the larva of the *Pronuba* moth, whose existence depends upon the destruction of some of the seeds. Theoretically, a single larva to each pod would give the optimum condition for *Yucca*, as this would represent a single pollination by a parent *Pronuba*. If the number of larvae be greater than one per pod, the advantage is on the side of the *Pronuba* until the number of larvae is such that the entire mass of seed produced is destroyed. This last condition would of course exterminate the moth.

That one larva to each pod is the optimum number is shown by the fact that 9 of the 11 pods in which but a single larva was found produced the average number of perfect seeds or more.

The averages also show that *Yucca* is successful in seed production even against odds. The average number of larvae per pod found was 3, and of the 26 pods with 3 or fewer larvae 12 produced

the average number of perfect seeds or more. That the presence of a number of larvae in a single pod need not prohibit average seed production is shown by plants 2 and 9. A pod of the former produced 172 perfect seeds, although 12 larvae had been in the pod. The pod of plant 9 also produced good seed in excess of the average even though there were 5 larvae in it. The number of larvae present did not seem to have any effect on the total number of seeds (both infertile and perfect) produced.

The production of the average number of perfect seeds by pods with many larvae calls attention to the influence of parasites of *Pronuba*. Of the 30 pods examined, 7 were found without injured seeds. In several other cases, although some of the seeds had been eaten, the dried skin of the larva was found in the burrow in the seed column where it had died. It is well known that most species of insects are parasitized by other insects, particularly by minute Hymenoptera. These parasites are usually parasites of the egg or the larva of the host. The action of such parasites would account for the death of the eggs and young larvae of *Pronuba*. The result of the presence of these parasites would be advantageous to *Yucca*, for once the parent *Pronuba* has pollinated the blossom of *Yucca* its value to that *Yucca* plant ceases. The destruction of seeds by larvae is unnecessary, as proven by 7 cases, and, so far as the particular plant is concerned, disadvantageous. The advantage comes only to the species as a whole, in supplying food for the *Pronuba* larva. The elimination of some of the eggs and larvae of *Pronuba* by parasites could also be of value to *Yucca* as a group, if not carried too far. These egg and larva parasites aid in preserving the balance by reducing the number of larvae toward the optimum for the plant, thus saving the plant the seeds eaten by the extra larvae. If the 7 pods which lost no seeds as the result of injury by larvae be dropped, the average number of seeds eaten per larva is raised from 19 to 24, and the number lost per pod from 58 to 72. The destruction of the egg in these 7 cases makes this important change in the averages, and if the saving of seed to the plant by the destruction of the immature larvae could in some way be figured in, the change would be still greater. Of course these eggs and larvae may not have been destroyed by parasites, although that is rather

improbable, judging by the cases of other insects, but the factor of elimination of some of the eggs and larvae remains, and the advantage, from whatever cause, is to the plant.

In conclusion it is to be noted that *Yucca* is a successful plant in regions where the climatic conditions are requisite and *Pronuba* is found. The peculiar method of pollination by a single insect species, which is maintained at the expense of the plant, must then also be considered successful. The averages show that it is. An average pod produces 300 seeds, over 100 of which are perfect, at a loss of 58 seeds, that is 21 per cent of the total production.

UNIVERSITY OF COLORADO  
BOULDER, COLORADO

## CURRENT LITERATURE

### BOOK REVIEWS

#### Trees in winter

In the past, tree manuals have either been without adequate illustrations or they have been too expensive for many who would otherwise have been students of tree life. The present volume<sup>1</sup> is inexpensive and yet each one of the more than 100 species described is represented by excellent halftone reproductions of photographs of the winter habit, the fruit, the twigs, and the bark, making in all more than 500 separate illustrations. The book consists of two parts, the first devoted to the planting and care of trees and covering 182 pages, and the second to descriptions of the tree species together with keys for their identification. The latter part, as it appeared in bulletin form, was reviewed in this journal<sup>2</sup> somewhat more than a year ago.

The introductory chapter on the study of trees discusses the importance of the subject for various grades of school and college work, as well as the aspects which appeal to the amateur, the artist, and the poet. Some directions are given for conveniently measuring the height of trees without elaborate apparatus, and tree photography is briefly considered. There follows a series of chapters on the selection of trees for various purposes, their planting, and their care. This topic is expanded to include the treatment of various injuries and the control of insect and fungous enemies.

The systematic part begins with a general chapter on the terms employed and their application to the description and identification of various species. It is followed by keys for (1) the genera and (2) the species. For the conifers, leaf and bud characters are employed; while for deciduous genera, the bud, leaf scar, and twig are made the principal bases of identification, with an occasional final appeal to the fruit. The description of species is concise, being limited to a single page for each, but quite sufficient for accurate determination, and is supplemented with data concerning the distribution, character, and economic uses of the wood, together with synonyms for both common and scientific names. Facing the description is the plate illustrating the tree, making the arrangement very convenient. A good index adds to the efficiency of this excellent work. Its scope fits it for use in the college, the school, and the home.—GEO. D. FULLER.

<sup>1</sup> BLAKESLEE, A. F., and JARVIS, C. D., *Trees in winter*. 8vo. pp. 446. *figs.* 103. *pls.* 109. New York: Macmillan. 1913. \$2.00.

<sup>2</sup> BOT. GAZ. 53:355. 1912.

### Local and spring floras

The increased demand for convenient and relatively inexpensive handbooks dealing with the flora of limited areas, particularly in the western states, has led to the publication of several local floras which deserve notice. Among these are: (1) PETERSEN'S<sup>3</sup> *Flora of Nebraska*, in which the author seeks to present a list of "all conifers and flowering plants, both native and introduced, which grow without cultivation in Nebraska." The enumeration of species is preceded by keys leading to their determination, and their distribution in the state is indicated. Formal descriptions, however, are omitted. (2) GARRETT'S<sup>4</sup> *Spring flora of the Wasatch region*, in which the author's aim is "to furnish a flora containing practically all the plants of a limited area that bloom during the spring months" or by the middle of June. The main object of this book is to stimulate the student to a study of the vernal flora of the "eastern edge of the Great Basin." The treatment of genera and species is conservative and reliable, and synonyms are introduced when clearness may be gained thereby. (3) NELSON'S<sup>5</sup> *Spring flora of the intermountain states*. In this book no pretense is made to include all the flowering plants of the spring season, but rather "some of the plants that bloom early in the year." It is intended to serve merely as an introduction to the flora of the region outlined, namely Colorado, Wyoming, Montana, and adjacent parts of Idaho, Oregon, and Utah; and in connection with it the author recommends the use of more complete manuals for reference.

These little volumes are of portable size, convenient for field work, and no doubt will be helpful to the student in gaining an introductory knowledge of the flora of the regions to which they appertain.—J. M. GREENMAN.

### Forests of Nova Scotia

In anticipation of adopting a definite policy of conservation of its forest resources, a survey of the existing conditions has been made by Nova Scotia.<sup>6</sup> The importance of such an undertaking is seen to be very great when it is realized that 80 per cent, or 14,000 square miles, of the province consists of non-agricultural land covered with forests, or fit only for that purpose, and that this resource, furnishing some five millions of dollars in value of annual

<sup>3</sup> PETERSEN, N. F., *Flora of Nebraska*. A list of the conifers and flowering plants of the state with keys for their determination. 8vo. pp. 217. Published by the author, Lincoln, Neb.: Printed by the State Printing Co. 1912.

<sup>4</sup> GARRETT, A. O., *Spring flora of the Wasatch region*. 2d ed. 8vo. pp. xii+139. Lancaster, Pa.: New Era Printing Co. 1912.

<sup>5</sup> NELSON, AVEN, *Spring flora of the intermountain states*. 8vo. pp. xv+204. Boston: Ginn & Co. 1912.

<sup>6</sup> FERNOW, B. E., HOWE, C. D., and WHITE, J. H., *Forest conditions of Nova Scotia*. Imp. 8vo. pp. v+93. pls. 12. maps 5. Commission of Conservation, Ottawa, Canada. 1912.

product, is in danger of exhaustion within the next two decades. Of the forest area less than 2 per cent is virgin, about 10 per cent is barren, while vast areas have been burned and are in various stages of reproduction. The pure deciduous forest includes less than 10 per cent of the whole, and the pure coniferous growth about 20 per cent, while the remainder is mixed, varying from a beech-maple-hemlock association, with a small percentage of spruce in the Annapolis Valley, to one in which birch, spruce, and balsam fir predominate in the more rugged portions of the province.

The ecological part of the survey was carried on principally by HOWE and his assistants. The geology and topography are carefully considered and related to the conditions of reproduction and distribution of the various tree species. Special attention is given to the causes and possibilities of the barrens and semi-barrens. Most of the latter are found to have been caused by repeated fires in regions with coarser soils. If the fires are excluded and provision made for seeding, HOWE concludes that within a century a marketable forest would be developed. All the conditions point not only to the need of conservation, but also to the comparative ease with which it could be instituted and the excellent results likely to follow such a policy.—GEO. D. FULLER.

#### MINOR NOTICES

**Syllabus der Pflanzenfamilien.**<sup>7</sup>—The student of taxonomy, as well as all those who have frequent occasion to use an abbreviated synopsis of the vegetable kingdom, will welcome the appearance of this new edition of the well known *Syllabus*. From former editions it differs mainly by the incorporation in the text of numerous carefully selected figures which greatly elucidate the subject. A few additions have been made and certain groups of plants which until recently have been placed doubtfully in the natural system are here given a definite position in the progressive sequence of orders. For example, the Julianiales (*Juliania* and *Orthopterygium*) is ranked as an independent order and placed between the Juglandales and Fagales in accordance with HEMSLEY's recent treatment. Manifestly, in a work of this scope it would be impossible to take cognizance of all genera known up to the time of publication; but a statement by these eminent authors of the relative position of the remarkable plant described as *Mitrastemon*, representing the monotypic order Mitrastemonales of MAKINO, would have been of interest to the systematist. However, this is but a slight omission when one considers that the purpose of the work is to serve as a convenient guide for the classification of plants in accordance with the most advanced knowledge of the science at the present time. The book contains a vast amount of authoritative information in epitomized form, and deserves, and doubtless will meet with, a wide circulation among American students.—J. M. GREENMAN.

<sup>7</sup> ENGLER, ADOLPH, and GILG, ERNST, *Syllabus der Pflanzenfamilien*. pp. xxxii+387. figs. 457. Berlin: Gebrüder Borntraeger. 1912.

**Terminology of experimental evolution.**—The more recent investigations of experimental morphology, experimental evolution, inheritance, and allied subjects have brought into existence a new technical vocabulary so extensive in its nature as to warrant such a work as the present volume by ROUX.<sup>8</sup> It is intended to supplement botanical, zoological, and medical dictionaries, duplicating them only in such terms as have been modified in their application, content, or implication by recent workers in the fields mentioned. It contains over 1000 terms, each defined and explained in considerable detail, and is made even more useful by citations to authors originating or employing the words, and by numerous references to terms of related concept. The work seems to be done in a manner which will realize the intention of the author, namely, to introduce the literature of these experimental studies to a wider circle of readers, to assist workers in the proper expression of their results, and to promote uniformity in a terminology which is necessarily at once somewhat extensive and rapidly enlarging.—GEO. D. FULLER.

**Geographical distribution of North American trees.**—The first of a series of atlases mapping the distribution of North American trees (exclusive of those confined to Mexico) has just appeared and is devoted to the genus *Pinus*.<sup>9</sup> It includes 36 maps, 14.5×20 inches, each giving in detail the range of one species of pine. In mapping these trees, all published records have been consulted and additional data have been drawn from the investigations and reports of members of the Forest Service. Unfortunately, it has been found impossible, because of lack of accurate detailed information, to indicate anything concerning the density or continuity of the growth of each species, hence only the botanical range is indicated. When completed this series of maps will form a valuable addition to present literature on North American forests.—GEO. D. FULLER.

**Queensland plants.**—The second edition of BAILEY's *Catalogue of Queensland plants*<sup>10</sup> is much enlarged and has been greatly improved by numerous illustrations, including 16 fine color plates. All the known plants of the state are listed, including the bryophytes and thallophytes. There are numerous notes on economic features, and the addition of vernacular and aboriginal names will be of great assistance to those who are looking for plants in localities where scientific names are unfamiliar. BENTHAM and HOOKER's *Genera Plantarum* and the *Flora Australiensis* have been followed for the spermatophytes

<sup>8</sup> ROUX, W., *Terminologie der Entwicklungsmechanik der Tiere und Pflanzen*. 8vo. ix+465. Leipzig: Englemann. 1912. *M*10.

<sup>9</sup> SUDWORTH, GEO. B., *Geographic distribution of North American trees*. Part I. Pines. U.S. Dept. of Agric. Forest Service. 1912.

<sup>10</sup> BAILEY, F. MANSON, *Catalogue of Queensland plants*. 8vo. pp. 879. figs. 976. colored pls. 16. Published by the Queensland Government. Brisbane. 1913.



and pteridophytes; for bryophytes and thallophytes the catalogue is based upon various monographs. BAILEY's experience with Australian plants, extending over half a century, adds much to the practical value of this work.—CHARLES J. CHAMBERLAIN.

**Plants of Palestine.**—DINSMORE<sup>12</sup> has published a catalogue of the plants of Palestine which is based on the well known floras of POST and BOISSIER, supplemented by additional collections made during the past few years. The catalogue includes the indigenous ferns, fern-allies, and flowering plants, also the cultivated plants of the region, and the total number of species amounts to about 2000. An interesting feature of the publication is the association of the Arabian name of the plant along with its scientific name. This part has been prepared by Professor G. DALMAN.—J. M. GREENMAN.

**Plants of Massachusetts.**—STONE<sup>13</sup> has published a list of the vascular plants of three counties of Massachusetts, which comprise the Connecticut Valley and represent a section of the state from Connecticut to New Hampshire and Vermont. This very interesting region has been the "stamping-ground" of such botanists as HITCHCOCK, TUCKERMAN, JESUP, CLARK, COBB, etc., and STONE has brought their work up to date. The list includes 75 pteridophytes, 16 gymnosperms, 417 monocotyledons, and 990 dicotyledons, 1498 species in all.—J. M. C.

**A manual of the cryptogams.**—ROSENGING<sup>14</sup> has published an account of the cryptogamic groups as a companion volume to the last edition of WARMING's *Systematic botany*, which includes only the seed plants. A wealth of material is presented, and more than 200 excellent figures (38 of them original) have been added to those that appear in the cryptogamic part of the older editions. As the author says, the volume is a handbook rather than a textbook, introducing students and teachers to material.—J. M. C.

**Dictionary of botanical names.**—ZIMMER<sup>15</sup> has prepared a small, compact dictionary which defines chiefly names of species. It will enable one who is not a linguist to discover what specific names really mean. The thought of the author is that it will give some interest to "these strange names that are all but barren of interest in themselves."—J. M. C.

<sup>12</sup> DINSMORE, J. E., *Die Pflanzen Palästinas*. Zeitsch. Deutsch. Palästina-Vereins 1911. Reprint pp. 122. Leipzig: In Kommission bei J. C. Hinrichs. 1911.

<sup>13</sup> STONE, GEORGE E., *A list of plants growing without cultivation in Franklin, Hampshire, and Hampden Counties, Massachusetts*. pp. vii+72. Amherst, Mass. 1913.

<sup>14</sup> ROSENGING, L. KOLDERUP, *Sporeplanterne*. pp. x+338. figs. 513. Copenhagen and Christiania: 1913.

<sup>15</sup> ZIMMER, GEORGE FREDERICK, *A popular dictionary of botanical names and terms*. pp. 122. London: George Routledge & Sons; New York: E. P. Dutton & Co. 1913. \$1.00.

## NOTES FOR STUDENTS

**Mitosis.**—In a remarkable series of papers on nuclear division, LAWSON establishes several claims which differ from current notions, and raises several questions which should stimulate research. The first paper, dealing with synapsis and showing that this is not a contraction phase, has already been noted in this journal.<sup>15</sup> The principal conclusion of the second paper<sup>16</sup> is that the achromatic figure is not an active factor in mitosis, but merely the passive effect of nuclear osmotic changes. Pollen mother cells of *Disporum*, *Gladiolus*, *Yucca*, *Hedera*, and the root tips of *Allium* furnished material for the investigation. In the pollen mother cells, after the great increase in the size of the nuclear vacuole during synapsis, the size of the nuclear vacuole begins to diminish. The nuclear membrane is not only extensible, but is elastic, so that the passage of karyolymph from the nucleus into the cytoplasm is accompanied by a diminution in the volume of the nucleus. When the nucleus has attained its greatest volume, the cytoplasm shows a reticulate structure; but as the size diminishes, the tension draws the network out into the familiar threads, known as kinoplasm, which become more and more distinct. When the nucleus has reached its minimum size, the nuclear membrane has always been reported to break down and disappear. LAWSON claims that in the living cell it does not break down at all. Even if there should be a rupture, the karyolymph, coming into contact with the cytoplasm, would immediately precipitate another membrane, so that no break would appear. However, breaks may occur in fixed material on account of osmotic changes due to the fixing agent, since the simultaneous killing of the cytoplasm would prevent the precipitation of a new membrane. When the stage is reached at which the membrane is reported to break down, the membrane is really so closely applied to the chromosomes that it is indistinguishable. The membrane being a part of the same cytoplasm which is drawn out into fibers, it is easy to understand why an approximately equal sheaf of fibers is attached to each end of each chromosome, for each chromosome is surrounded by a part of the nuclear membrane which forms the base of the fibers. The shifting of fibers and cones of fibers, so frequently described, does not mean that individual threads or cones travel through the cytoplasm, but rather that new lines of tension are developed, so that what was formerly reticulate cytoplasm becomes fibrillar, and what was fibrillar resumes the reticulate form. The cones of the multipolar spindle do not fuse to form a bipolar spindle, but the bipolar spindle is the expression of a new line of tension. The spindle in vegetative cells does not assume the multipolar form on account of the vacuolate cytoplasm, pollen mother cells being practically free from vacuoles.

<sup>15</sup> BOT. GAZ. 51:313. 1911.

<sup>16</sup> LAWSON, A. A., Nuclear osmosis as a factor in mitosis. Trans. Roy. Soc. Edinburgh 48:137-159. pls. 1-4. 1911.

In the third paper,<sup>17</sup> dealing with chromosome reduction, the material used was *Smilacina*, *Kniphofia*, and *Aloe*. LAWSON finds no continuous spirem at any stage, but always a number of separate threads, probably as many as there are chromosomes. Even in the reticulum the threads are double and the double character becomes more pronounced in later prophase. If these two parts of each chromosome should separate at this stage, an ordinary vegetative division with the diploid number of chromosomes would result; but, as the threads shorten and thicken, the double character becomes indistinguishable, so that each thread appears single. These apparently single threads now unite laterally in pairs, forming bivalent chromosomes, and the two members of the bivalent chromosome are separated at the heterotypic mitosis, so that entire vegetative chromosomes pass to the pole, thus accomplishing the reduction in number. Although the conclusions are contrary to generally accepted views, the figures and arguments seem convincing.—CHARLES J. CHAMBERLAIN.

**Changed permeability and antagonism.**—During the last half-decade LEPESCHKIN, TRÖNDLE, and other workers have developed accurate methods for determining the rate at which various solutes (NaCl, KNO<sub>3</sub>, glycerine, glucose, etc.) enter the plant cell. LEPESCHKIN states the degree of permeability in the following unit: molecular weight entering unit surface of the cell in unit time per average mol difference in concentration inside and outside the cell. These methods have been applied in determining the effect of various conditions and reagents upon the permeability of the protoplasm to the solutes studied. The work as a whole establishes that marked changes in permeability to nutrient salts and other solutes are produced by variations in temperature or light-intensities and by the application of anesthetics or certain salts. It also involves definite measurement of the magnitude of the permeability changes and leads to the generalization that in nature the protoplasm of plant cells changes in its degree of permeability from hour to hour with the changing condition, and that there exist daily, seasonal, and annual rhythms of permeability changes. It is rather hard to over-emphasize the physiological significance of these facts in explaining some phases of plant activities. For example, LEPESCHKIN has found that variation movements in plants are caused in the main by modified permeability of pulvinal cells to contained solutes, which is induced by changing environment or internal conditions.

Now SZÜCS<sup>18</sup> believes he has shown that the antagonistic action of various metallic ions toward other metallic ions, alkaloids, and basic dyes is due to the antagonistic ions reducing the rate at which the toxic agents mentioned enter

<sup>17</sup> LAWSON, A. A., A study in chromosome reduction. Trans. Roy. Soc. Edinburgh 48:601-627. pls. 1-3. 1912.

<sup>18</sup> SZÜCS, JOSEPH, Experimentelle Beiträge zu einer Theorie der antagonistischen Ionenwirkungen. Jahrb. Wiss. Bot. 52:85-142. 1912.

the protoplasm. He first makes a study of the antagonism of  $\text{AlCl}_3$  to  $\text{CuSO}_4$ . For this study he uses the hypocotyls of *Cucurbita Pepo*, which are placed in the solution to be tested for a given time, removed, rinsed with distilled water, and dried with filter paper, then placed in a horizontal position in saturated air, and after 24 hours examined for geotropic response. The length of exposure at a standard temperature in a given solution that nulls geotropic reaction in 70 per cent of the hypocotyls is termed the life-duration for the solution. In 0.025  $n$   $\text{CuSO}_4$ , the life-duration is less than 40 minutes; but if the solution also contains 0.15  $n$   $\text{AlCl}_3$ , the life-duration is 4 hours. In this concentration of  $\text{CuSO}_4$ , higher or lower concentrations of  $\text{AlCl}_3$  give shorter life-durations. In 0.005625  $n$   $\text{CuSO}_4$ , the life-duration is about one hour, but if  $\text{AlCl}_3$  is present in 0.025  $n$  concentration, the life-duration is 22 hours; and if in 0.07  $n$  concentration, 26 hours. The author shows that in a given  $\text{CuSO}_4$  solution for a given time far less copper enters the hypocotyl when  $\text{AlCl}_3$  is present, but it is to be regretted that his determinations of copper were not quantitative. It is maintained that the slower entrance of the copper salt in the presence of  $\text{AlCl}_3$  is due to a lowering of the permeability of the plasma, as such, to the former, and not to the other possibility of lowered toxicity, for concentrations of  $\text{AlCl}_3$  that are themselves quite injurious to the cells lower markedly the rate of entrance of  $\text{CuSO}_4$ . Potassium nitrate showed some antagonism against the toxicity of quinine hydrogen chloride and methyl violet to *Spirogyra*. The nitrate of calcium was much more effective in this respect, and it in turn was greatly excelled by the nitrate of aluminium. In these cases also antagonistic action seems to be due to reduced permeability. Inorganic salts are not constantly antagonistic to the toxic action of piperidine on *Spirogyra*; while some increase the toxicity, others lower it. The effect in these cases is a function of both the kation and the anion, although the kations are predominant in their influence. Slight traces of alkaloids and basic dyes in the protoplasm render it more subject to deformation by the salts used, whether the latter act antagonistically or not.—WM. CROCKER.

**Wild wheat in Palestine.**—The discovery of wild wheat in Palestine by AARONSOHN has attracted a great deal of attention, chiefly because of the possible practical importance of a hardy race of wheat. This Palestinian wheat has now been under observation and culture for three or four years, and the general results have been summarized by COOK<sup>29</sup> in a bulletin of the Bureau of Plant Industry. The whole bulletin is of interest, but only certain general conclusions can be selected for mention.

The wild wheat was discovered on Mount Hermon, but later it was found growing under very different conditions in the Jordan Valley, and probably has a much wider range. It is especially abundant on limestone formations,

<sup>29</sup> COOK, O. F., Wild wheat in Palestine. Bull. 274, Bur. Pl. Ind., U.S. Depart. Agric. pp. 56. figs. 11. pls. 15. 1913.

being absent or very scantily represented on volcanic intrusions. It is exceedingly variable in pollination methods, being not only adjusted for cross-pollination by the extrusion of the stamens, but in some cases being protogynous or protandrous. Occasionally it is adjusted for self-pollination, like the domesticated wheats. It is thought that the remarkable individual diversity shown by this wild wheat is explained by the great freedom in adjustments for pollination. It is further concluded that the self-pollination of the domesticated races is not a primitive condition, but that the adjustments for cross-pollination have been lost, and as a consequence there has been a decline in vigor, fertility, and disease-resistance.

The joints of the rachis are peculiar in separating from one another at maturity, each joint remaining attached to its spikelet and forming a barbed beak. It was observed that these beaked spikelets "creep into crevices of rocks or bury themselves in the soil."

There seems to be no reason to doubt that this Palestinian plant is a genuine wild wheat, but it is by no means certain that it is the prototype of our domesticated races. In fact, it is suggested that it be named as a distinct species (*Triticum hermonis*, from Mount Hermon). Of course, whether it is the prototype of our domesticated races or not is not a question that affects its practical value.

*T. hermonis* is a hardy plant in the sense of being able to live under a wide range of natural conditions, and it suggests the possibility of obtaining from it races of wheat adapted to the arid regions of the southwestern states. There is also a possibility of breeding its rust-resistance into our domesticated races. It is even suggested that this wild wheat may be used as a self-sown forage plant on the grazing lands of the Southwest; but the caution is urged that it might become a troublesome weed!—J. M. C.

**Biologic species of *Rhytisma*.**—The widespread occurrence of *Rhytisma acerinum* on many species of maple has led MÜLLER<sup>20</sup> to investigate the host relationships of this form whose distribution seems to indicate that it includes several biologic species. The field observations which led to this view were fully confirmed by cultural experiments which showed that the forms usually included in *Rhytisma acerinum* can be separated into at least two biologic species. The name *R. acerinum* is retained for one of these, the other is described as *R. pseudoplatani*.

Under *R. acerinum* two minor forms are distinguished according to the ease with which they infect different species of maple. *R. acerinum* f. *platanoides* occurs principally on *Acer platanoides* and infects less easily *A. pseudoplatanus* and *A. campestre*. *R. acerinum* f. *campestre* occurs principally

<sup>20</sup> MÜLLER, K., Zur Biologie der Schwarzfleckenkrankheit der Ahornbäume, hervorgerufen durch den Pilz *Rhytisma acerinum*. Centralbl. Bakt. II. 36:67-98, figs. 4, pls. 4. 1912. Preliminary account, Ber. Deutsch. Bot. Gesells. 30:385. 1912.

on *A. campestris*, and less abundantly on *A. platanoides*, but not at all on *A. pseudoplatanus*. *R. pseudoplatani* infects only *A. pseudoplatanus*, upon which it occurs in company with *R. acerinum platanoides*.

Infection by the spores of *Rhytisma* always takes place on the lower surfaces of the leaves. Observations during a period of six years show that there is a parallelism between the severity of the disease and the quantity of rainfall. No appreciable injury results to the trees from the attack of the fungus.

An experiment carried out by LESLIE and reported by TUBEUF<sup>21</sup> confirms in part the results of MÜLLER. Leaves of *Acer pseudoplatanus*, bearing sclerotia with ripe ascospores of *Rhytisma*, were suspended over pot-plants of *A. pseudoplatanus*, *A. platanoides*, *A. campestre*, and *A. Negundo*. Of these only *A. pseudoplatanus* was infected, showing that the fungus was unmixed *R. pseudoplatani* of MÜLLER.—H. HASSELBRING.

**Fertilization in *Lilium*.**—Although every cytologist has studied fertilization in *Lilium* and many have published their observations and conclusions, BLACKMAN and WELSFORD<sup>22</sup> have added a short paper to the list. They confirm the observation of NAWASCHIN and others, that the male nuclei are not accompanied by any cytoplasm, and agree that the male nuclei are capable of independent movement. The male nucleus which fuses with the polar nuclei is larger than the one which fuses with the egg nucleus, and is distinctly pointed. In both nuclei the chromatin is not in the form of a resting network, but is arranged in strands resembling a spirem. NAWASCHIN thought this arrangement was due to the motile condition, but the present authors suggest that it represents an early prophase of division. In *Fritillaria*, and perhaps in *Lilium*, the pollen tube does not enter the cytoplasm of the sac, but presses between the cytoplasm of the egg apparatus and the cytoplasm of the middle portion of the sac.—CHARLES J. CHAMBERLAIN.

**Nuclei in sieve tubes.**—It is generally accepted that the nuclei of sieve tubes soon disorganize and disappear, even such investigators as STRASBURGER (1891) and ZACHARIAS (1895) having failed to find nuclei in the mature tubes. By using the careful methods of modern cytology, SCHMIDT<sup>23</sup> finds that in *Cucurbita Pepo*, *Victoria regia*, and *Trapa natans*, the nuclei can always be demonstrated, even in the older sieve tubes. The technique of 1891 and 1895 was sufficient for the demonstration of these nuclei, but apparently it was not thought worth while to use such tedious methods in anatomical work. SCHMIDT promises a paper on the structure and function of the sieve tube.—CHARLES J. CHAMBERLAIN.

<sup>21</sup> TUBEUF, K. VON, Rassenbildung bei Ahorn-*Rhytisma*. Naturwiss. Zeitschr. Forst- u. Landwirtschaft. 11:21-24. 1913.

<sup>22</sup> BLACKMAN, V. H., and WELSFORD, E. J., Fertilization in *Lilium*. Ann. Botany 27:111-114. pl. 12. 1913.

<sup>23</sup> SCHMIDT, E. W., Der Kern der Siebröhre. Ber. Deutsch. Bot. Gesells. 31:78, 79. 1913.

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BOTANICAL GAZETTE

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THE ORIGIN AND DEVELOPMENT OF THE EMBRYO  
SAC AND EMBRYO OF DENDROPHTHORA  
OPUNTIOIDES AND D. GRACILE. I<sup>2</sup>

HARLAN HARVEY YORK

(WITH SIX FIGURES AND PLATES V AND VI)

## Introduction

The Loranthaceae are parasitic plants that occupy a place of special interest among the dicotyledons. Since they are very peculiar, not only in their mode of life, but also in the structure of their reproductive organs, they have attracted much attention from plant morphologists. Although a considerable number of studies of the group have been made, their morphology and physiology are quite inadequately known. Our knowledge even of the European species of *Loranthus*, *Arceuthobium*, and *Viscum*, those that have been studied most, is as yet incomplete.

The Loranthaceae comprise more than 600 species and are confined largely to the tropics. Aside from the European species of the genera just mentioned, the development of these plants has been most fully studied in certain species of the same genera occurring in Java. In spite of the wide distribution of the genus *Phoradendron* in the southeastern United States and the occurrence of scores of other species in tropical America, comparatively little is known of the Loranthaceae of the Western Hemisphere.

<sup>2</sup> Contribution from the Botanical Laboratory of the Johns Hopkins University, no. 26.

The observations of the earlier writers on the reproductive organs of the Loranthaceae were concerned chiefly with the mature flowers and the development of the fruit. Their interpretation of the different floral organs and the relation of these to each other show that they had a very meager understanding of these structures. Nevertheless, some of these observations show considerable accuracy and are therefore worthy of mention.

WILLIAM GRIFFITH (10), in a paper read before the Linnaean Society of London in 1836, gave a brief description of the anatomy of the flowers and the development of the embryo in *Loranthus scurrula*. He asserted that the ovary is "intimately adherent" with the calyx and that some time after the "fall of the corolla a small cellular body appears attached at the base of the ovarian cavity." This structure, which is an elongation of the floral axis, he interpreted as the rudiment of an ovule, at the center of which, in later stages of development of the fruit, an embryo appeared. In a second paper read before this same society in 1843, GRIFFITH (11) described the mature embryo sacs of *Loranthus bicolor* and *Loranthus globosus*, stating that he believed they existed even before pollination. He found a "nipple-shaped process" at the base of the ovarian cavity and thought it might be a continuation of the floral axis. Not being sure of the homology of this process he limited his descriptions to the ovules, which he called embryo sacs, their relations to the pollen tubes, and the subsequent changes in them. His main conclusions are that the ovules in this genus are reduced to embryo sacs, and that "the embryo is a growth from the ends of the continuations of the pollen tubes, outside the anterior ends of the embryo sacs."

SCHLEIDEN (31) regarded the flowers of the Loranthaceae, which he studied in *Viscum album* and *Loranthus* sp., as the "simplest that can exist." He asserts that the two pairs of bracts of the perianth which bear the stamens are "metamorphosed into anthers" and the segments of the perianth in the carpellate flower "have the nature of a calyx." According to him the floral axis is prolonged between the sepals, forming a nucellus or "ovulum nudum." Furthermore, the tip of the nucellus constitutes a stigma, on which the pollen grains are deposited and into the tissue of which



the pollen tubes, containing the "germ of the embryo," grow downward to the embryo sacs, in which the embryos are nourished. He believed that the embryo sacs are formed in the "pith of the peduncle" or the center of the nucellus, and that the berry is to be regarded as a "metamorphosed peduncle." In conclusion, SCHLEIDEN states that "the Loranthaceae show, in a parasitic form, the intervening step between the Coniferae and more highly developed families" of the angiosperms. MEYEN (21) held essentially the same views as SCHLEIDEN.

TREVIRANUS (36) disagreed with SCHLEIDEN and MEYEN, believing that the central portion of the flower of *Viscum album* constitutes an ovary, within the solid tissues of which the embryo sacs arise.

The investigations of HOFMEISTER (13) greatly advanced our knowledge of the floral organs of the Loranthaceae, he being the first to study the origin and development of the flowers. His extremely accurate observations were made on *Loranthus europaeus* and *Viscum album*. In these forms the flowers are axillary in origin and position. According to HOFMEISTER, soon after the sepals and carpels have appeared in *Loranthus* the apex of the floral axis elongates, growing up between the carpels and forming a small cone-shaped mass of tissue which later becomes united with them. He found that the embryo sacs are formed in the elongated floral axis which he regarded as "a naked ovule." Below this "ovule" is a little plate of collenchymatous tissue which he believed to be chalazal in nature. He also found that the floral parts in *Viscum album* originate quite similarly to those in *Loranthus europaeus* except that there is no swelling of the floral axis between the carpels, which finally fuse to form a single mass of tissue. The embryo sacs, which are usually two in number, arise from a group of cells in the tip of the floral axis.

VAN TIEGHEM (37), from his observations on *Viscum album*, was led to believe that the central mass of tissue of the flower is apical in origin and homogeneous throughout its extent. He described it as being formed by two carpellary leaves, each having its own vascular supply from the peduncle, and becoming "connate" on their "ventral surfaces." He believed that embryo sacs arise

in the lower half of the carpellary tissue, one or two sacs for each carpel. He interpreted the central parenchymatous tissue as a solid ovary, no special segments being differentiated for the production of ovules.

We are indebted chiefly to TREUB (33) for an accurate account of the development and a clear interpretation of the reproductive organs of the Loranthaceae. He found in *Loranthus sphaerocarpus* that the tip of the floral axis grows up between the carpels, forming a mammilliform body, the "mamelon," which becomes united to them along their inwardly projecting margins. Between these lines of union with the carpels the "mamelon" is lobed. There are as many lobes as carpels, which are 3-5 in number. An embryo sac is formed in each lobe of the "mamelon." TREUB interpreted these outgrowths of the "mamelon" as rudimentary ovules, and the central region of the "mamelon" he regarded as a placenta. He found in *Loranthus pentandrus* but a slight elevation of the floral axis between the carpels which he called a "rudimentary placenta" and in which the embryo sacs are formed. TREUB (35) described a still greater reduction in the floral parts of *Viscum articulatum*, in which there is no projecting placenta or "mamelon." The embryo sacs in this species arise from sub-epidermal cells of the sunken apex of the receptacle. JOST (15) described later a similar condition in *Viscum album*.

According to JOHNSON'S (14) account of his observations on *Arceuthobium Oxycedri*, there is at the time of pollination a projection of the floral axis which fills the entire ovarian cavity but does not fuse with its walls. He has shown that in opposite sides of this body two embryo sacs are formed, each of which arises from a hypodermal cell. JOHNSON assigns the same morphological value to the elongated axis that TREUB did for *Loranthus sphaerocarpus*. He describes the anthers as sessile on the segments of the perianth and without vascular bundles.

A few years later, PEIRCE (24), in his studies on *Arceuthobium occidentale*, confirmed JOHNSON'S account of the morphology of the fruit. His observations together with those of YORK (43) on *Phoradendron flavescens* are the most detailed which have been made on the fruit of the American Loranthaceae.

During May and June of 1910, observations were made on 18 different species belonging to 8 different genera of Lorantheaceae occurring in Jamaica, and material was collected for a detailed study of their development. From a part of this material the following study was made. This paper, which embodies the results of the beginning of a comparative study of the morphology and physiology of the North American Lorantheaceae, embraces an account of the origin and development of the embryo sac and embryo of *Dendrophthora opuntoides* and *D. gracile*, together with a discussion of the physiological relation existing between the gametophyte and sporophyte of these plants. Although this study may lead to the extension of our knowledge of the life histories and relationships of certain genera of the Lorantheaceae, and perhaps to a clearer understanding of the systematic position of this family, one of the most interesting as well as most difficult problems for solution will be the determination of the factors which have caused the striking modification and adaptations in the reproductive organs. The material on which this study was made was killed in medium chromacetic acid and cut in sections  $10\mu$  in thickness.

To Professor DUNCAN S. JOHNSON the writer wishes to express his sincere thanks and appreciation for helpful criticism and advice.

#### **Dendrophthora opuntoides**

*Dendrophthora opuntoides* (L.) Eich. receives its name from its *Opuntia*-like appearance (text fig. 1). It is a bright yellowish green, glabrous shrub, seldom more than 4 dm. in height. The stems are jointed, constricted at the nodes, and all the joints of a branch are strongly flattened in one plane. The secondary branches arise in this plane in the axils of the reduced scalelike leaves at the nodes. The internodes are usually paddle-shaped, tapering toward the base. The flowers are opposite, isolaterally arranged in spikes which arise from the nodes of the younger portions of the stem in the same manner as the vegetative branches. The spikes thus formed are strongly flattened structures, whose plane of flattening is parallel with that of the stems. The position of a branch as it develops may vary somewhat from the plane in which the stems are flattened, so that all parts of the whole plant do not always lie

in one plane. However, the whole plant is essentially a single isolateral system.

*D. opuntiioides* as observed in Jamaica occurs in well lighted positions at elevations of 2000-5500 ft. above sea-level. It was found most frequently on the following named plants: *Oreopanax*



FIG. 1.—*Dendrophthora opuntiioides*: h, branch of host

*capitatum* Decne. and Planch., *Rhytidophyllum tomentosum* Mart., *Baccharis scoparia* Sw., *Heterotrichum patens* DC., and *Byrsonima* sp.

#### *Dendrophthora gracile*

*Dendrophthora gracile* Eich. is dark yellowish green in color and is quite similar in appearance and mode of branching to *D. opuntiioides*. The branches, however, are often almost terete, and the tendency toward an isolateral arrangement of the branches is less prominent than in *D. opuntiioides*. The flowers may be

arranged as in the latter, but they often show a tendency toward a decussate or whorled arrangement. The plants are distinctly dioecious, both forms being exactly alike in external appearance, except that the staminate flowers are usually more densely crowded on the spikes. The anthers are unilocular and sessile on the segments of the perianth. *D. gracile* was found at altitudes of 5000-6500 ft., in well exposed places. It was observed mostly on *Vaccinium meridionale*.

### The spikes

Since these two species of *Dendrophthora* are so much alike, the following descriptions, which are drawn primarily from *D. opuntiioides*, will be understood to apply to both unless otherwise stated. Each spike is usually composed of two or three internodes, and at the base of each internode there is a pair of connate bracts (figs. 3, 6). Primary growth of this axis is accomplished by the activity of an apical group of initials (fig. 2). There is considerable secondary elongation of the spike, due to an intercalary growth zone at the base of each internode.

A single pair of flowers is usually borne in each internode with the exception of the basal one (fig. 3). They arise laterally just below the apex of the spike, and are at no time closely associated with the bracts at the base of the internode, that is, they do not seem to be axillary in position (figs. 4, 5). In other spikes, which occur less frequently, there are often two or three pairs of flowers on each internode; of these the terminal pair is evidently older, and if three pairs are present, the basal pair is youngest of all (fig. 6). The appearance of these internodes, when the flowers are nearly mature, suggests that the second and third pairs have arisen successively during the intercalary growth of the internode, and that they have arisen from the young tissue at the base of the internode while this portion was still surrounded by the subtending bracts. This phenomenon, which seems unusual in these species, was noted by EICHLER (7) in *Dendrophthora Mancinellae* Eichl., and is a characteristic feature, for example, of the inflorescence of *Phoradendron crassifolium* Pohl., where the number of flowers on each internode is much greater than in *D. opuntiioides*.

The vascular system of the spike is comparatively simple. From each spike there enter the stem 6-14 vascular bundles, which are arranged as in a typical dicotyledonous stem except that there is no interfascicular cambium (text fig. 7). Growth in thickness is entirely by the divisions of the fascicular cambium and the parenchyma cells between the bundles. A single leaf trace passes from each segment of the perianth into the axis of inflorescence. The paths of the bundles were traced in detail through the basal internode, the first node above the stem, and the first pair of flowers. One bundle, which is entirely free from the others, extends from each of the two connate bracts into the axis. The first pair of bundles on the right and left sides of the axis

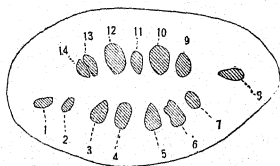


FIG. 7

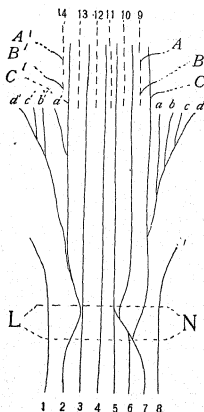


FIG. 8

FIGS. 7, 8.—Fig. 7, cross-section near base of spike, indicating arrangement of vascular bundles; fig. 8, diagram showing arrangement of vascular bundles in lateral view in the first node and first pair of flowers of fig. 3: 1, 2, 3, 4, 5, 6, 7, 8, same as in fig. 7; LN, level of first node; 1, 8, bundles entering the bracts; a, b, c, d, a', b', c', d', tracheid strands entering the swollen portion of the inflorescence axis (fig. 4, c) about flower; A, B, C, A', B', C', bundles entering inflorescence axis from the flower (figs. 13, v, 20, v); 9, 10, 11, 12, 13, 14, as in fig. 7.

furnishes the traces for the swollen collar of the stem surrounding the flower. With these same bundles the vessels of the flowers are connected (text figs. 7, 8). The bundles beyond the first pair of flowers were not followed closely, but their distribution is apparently similar to the portion of the system here figured and described.

### The flower

The earliest stage obtained in the development of the flower shows that it is not axillary in origin, but is initiated by a bulging out of the periblem of the inflorescence axis some distance above the axil of the subtending bract (figs. 4, 5). The flowers of *Rhopalocnemis phalloides* have a similar origin, as was shown by LOTSY (20). Following such a stage, the surrounding tissues of the incipient floral axis expand rapidly, which results in it becoming almost completely buried within the axis of the young spike (figs. 4, 9). Two cycles of floral segments develop acropetally on the young axis of the flower. The outer consists of three parts, which are the segments of the perianth; while the inner is composed of two segments, the carpels. The perianth completely covers the apex of the floral axis before the carpels have begun to develop (fig. 10). The two carpels arise as distinct primordia, and upon elongating inclose the moundlike apex of the floral axis between them (fig. 11). At this period of development the flower is still sunken in the axis of the spike, and its various parts are clearly distinct from one another (fig. 12, text fig. 12a). The apical growth of the floral axis is never active, and the outer region, composed of torus and the basal portion of the carpels, now grows rapidly, and thus the axis with the narrow ovarian cavity about it becomes deeply sunken within the torus (fig. 13). According to GOEBEL (9), there is a somewhat similar uprising of the torus and carpels

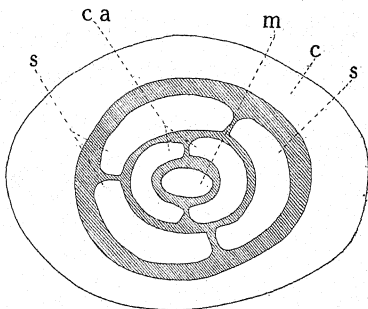


FIG. 12a.—Diagrammatic cross-section of flower taken at AB, fig. 12: *m*, floral apex or "mamelon"; *ca*, carpels; *s*, sepals; *c*, swollen part of inflorescence axis about flower.

in the early development of the flower of *Pyrus Malus*. The lateral walls of the ovarian cavity are thus lined on the inside by carpellary tissue and the ovary is distinctly epigynous. Meanwhile the moundlike apex of the floral axis has been slowly developing and has come to occupy the entire ovarian cavity. It is in close contact with the walls of the cavity but never becomes united with them. In form, it is a compressed knob, flattened in the same plane with the spike. A transverse mid-section of the knob is oval in outline (fig. 14). A longitudinal section perpendicular to its broad surface is finger-shaped, while a longitudinal section in the plane of flattening shows the knob form (figs. 15, 17, 18).

HOFMEISTER in *Loranthus europaeus* and TREUB in *L. sphaerocarpus* found that the floral axis elongates between the carpels after they have appeared. According to BAILLON (1), the apical part of the floral axis is present throughout the development of the carpellate flower in *Arceuthobium Oxycedri*, just as we have seen it to be in *Dendrophthora*.

#### The megasporangium (nucellus?)

The floral apex is composed entirely of parenchymatous tissue and has a distinct epidermis. Parallel with the enlargement of the floral axis the cells subjacent to the sporogenous cells divide by periclinal and anticlinal walls (fig. 16). Later, when the uninucleate sac has been formed, it is partially surrounded by a tissue of a few cells in thickness, which has resulted from the divisions of these underlying cells. The contents of the cells of this tissue contain no starch, but have dense cytoplasm, their walls are thicker, and they stain more darkly than the cells of the other portions of the axis (fig. 17). The tissue thus formed about the young gametophyte is apparently nutritive in function, and may be regarded as the equivalent of a nucellus. TREUB has shown that a similar tissue is formed about the archesporial cells in *Loranthus sphaerocarpus*, but the cells are in this case filled with starch. In *Arceuthobium Oxycedri* there is apparently no nucellus formed as in the above mentioned cases. By the time the two-nucleate sacs are developed, the cells of the nucellus have become much enlarged and the axis has become distinctly lobed (figs.



17, 18). A similar though somewhat more marked lobing occurs in *Loranthus sphaerocarpus* (TREUB 33). The epidermal cells immediately above the sporogenous cells divide by one or two periclinal walls, forming a small cap of cells which is probably to be regarded as the remnant of an integument (fig. 18). WARMING (38), in studying the development of the reproductive organs of *Thesium*, found that the contents of the epidermal cells above the apex of the nucellus were more densely granular than the remaining epidermal cells, and that the epidermal cells surrounding these granular ones divide by periclinal walls, forming a tissue about three cells in thickness, which he regarded as vestiges of integuments.

The question of the interpretation of the elongated floral axis, the "mamelon," has been a puzzling one. According to HOFMEISTER, who first worked out its development in *Loranthus europaeus*, it is a "naked ovule" in which there are several groups of archesporial cells present. BAILLON also gave the same interpretation to this body in *Arceuthobium*. TREUB believed that the "mamelon" is a growth of the floral axis in which the separate nucelli represent rudiments of ovules. In reference to HOFMEISTER'S idea, TREUB asserts that there is no reason to consider this hemispherical process as an ovule reduced to its nucellus. Nowhere are groups of embryo sac mother cells formed in the lateral part of a nucellus as would be the case in *Loranthus* if the "mamelon" be regarded as an ovule.

Aucune raison ne nous engage à considérer le processus hémisphérique comme un ovule réduit à son nucelle. Nulle part plusieurs groupes de cellules mères de sacs embryonnaires ne naissent dans les parties latérales d'un nucelle, comme cela serait le cas chez le *Loranthus* si le mammelon en litige méritait le rang d'ovule.

Comparing the enlarged floral apex or "mamelon" as seen in the Loranthaceae with that in certain genera of the Santalaceae, we find a striking resemblance and further evidence for the correctness of TREUB'S views. In the early development of the flower of *Thesium divaricatum*, there is a central elongation of the floral axis as in *Dendrophthora opuntioides* and *D. gracile*. This "mamelon" elongates with the formation of the ovarian cavity and forms a

lobe opposite each of the three carpels. As development continues, these lateral outgrowths enlarge and grow downward along the sides of the axis. These lobes are the ovules. In direction of growth they are anatropous and do not become fused with the placenta along the side of which they grow. Only mere rudiments of integuments are formed, as has been already mentioned. The ovules are thus practically naked. The archesporial cells are subepidermal in origin. In *Santalum album* there is a conelike "mamelon" quite similar to that in *Thesium*, but less extensively lobed. The ovules are without integuments. In *Osyris alba* naked ovules are found on a central placenta as in the two preceding examples, except the ovular lobes at first grow downward and then curve upward toward the apex of the placenta.

From this comparison it is evident that TREUB's interpretation of the "mamelon" is correct. The two lateral lobes of the elongated floral axis in *Dendrophthora opuntioides* and *D. gracile* are thus rudimentary ovules borne on a central placenta. The micropylar end of the nucellus is toward the base of the placenta, and the chalazal portion lies in the apical part of the floral axis (fig. 17).

#### The vascular system of the flower

One vascular trace passes from each of the three segments of the perianth into the inflorescence axis. A fourth vessel may enter the axis from near the base of the placenta, but there are no traces in the placenta. The bundles within the segments of the perianth as a rule never branch. About the time of the origin of the one-celled embryo sac, the cells just below the insertion of the placenta begin to enlarge and the walls become unequally thickened. Later there is formed a mass of short tracheids, which are somewhat similar in appearance to water-storage tracheids (fig. 19). This plate of tracheids is analogous to the plate of collenchymatous tissue in *Loranthus europaeus* which HOFMEISTER interpreted as being chalazal in nature. TREUB has shown that a collenchymatous body similar to that described by HOFMEISTER is present in *Loranthus sphaerocarpus*. During the formation of these short tracheids, a series of longer tracheids appear in the outer wall of the ovary at the level of the base of the placenta (text fig. 20, *t*). They

originate thus in or near the zone of elongation of the torus and extend upward from their place of origin, finally fusing with the bundles in the segments of the perianth. Other strands of tracheids pass upward from the plate of tracheids (text fig. 20, *tp*) near the

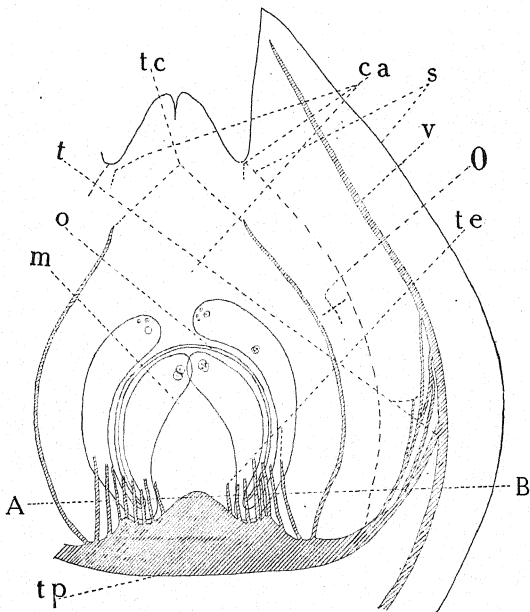


FIG. 20.—Diagrammatic longitudinal section of flower at maturity of embryo sac; *te*, strands of tracheids in inner walls of carpels; *t*, strands of tracheids connecting vascular bundles of the sepals with tracheid tissue beneath "mamelon"; *v*, vascular bundle of sepal; *tp*, plate of water tracheid tissue beneath "mamelon"; *tc*, strands of tracheids in middle region of carpels; *s*, tissue of sepal; *ca*, tissue of carpels; *m*, "mamelon"; *o*, cavity of ovary.



are first distinguished by their larger nuclei and more densely staining protoplasm, and without cutting off tapetal cells they develop directly into what may be called megaspore mother cells (fig. 16).

JOHNSON'S account of *Arceuthobium Oxycedri* states that the primary archesporial cell divides into two cells. The upper one becomes the primary tapetal cell, which later divides by an anticlinal wall into two cells, while the lower cell becomes "the mother cell of the embryo sac." TREUB'S work shows that no tapetal cells are formed in *Loranthus sphaerocarpus*. He found that the archesporial cell in *Viscum articulatum* does divide into two cells, the lower one developing directly into an embryo sac. It is not possible to determine the character of the upper cell, since it is not known where reduction division occurs.

Preceding division the megaspore mother cell becomes very much enlarged (fig. 16). Later the chromatin thread is organized, becomes thickened, and forms a loose mass, the synaptic knot. Following synapsis, it segments into chromosomes, which later become arranged on the spindle. From two counts of the chromosomes in the dividing megaspore mother cell nuclei of two different ovules at this stage of development, it is apparent that 18-20 chromosomes pass to each pole of the spindle. The same number of chromosomes was found in dividing cells of the young embryo. Thus it seems that no reduction division takes place; hence this division is a normal vegetative division. Since the nucleus passes through what is apparently a synapsis before dividing, it may be regarded as analogous to a megaspore nucleus of the usual type. The two cells resulting from the division of the so-called megaspore mother cell in *Dendrophthora* are separated by a thin wall and lie just beneath the epidermis of the nucellus (fig. 24). The one toward the micropylar region of the nucellus degenerates, while the one in the chalazal portion gives rise to the embryo sac (fig. 25).

In the seed plants in which parthenogenesis is said to occur there is a tendency toward a reduction of the number of divisions of the so-called megaspore mother cell. Four megaspores are formed in *Thalictrum* and *Eualchemilla*. Only two occur in *Taraxacum*

and *Hieracium*, while in *Antennaria* and *Wikstroemia* they have been omitted entirely. The archesporial cell develops directly into an embryo sac in *Balanophora elongata* and *Elastonema acuminatum* investigated by TREUB, and in *Balanophora globosa* studied by LOTSY. The authors claim that the embryo develops apogamously.

In *Arceuthobium Oxycedri*, according to JOHNSON, two small cells are cut off from the lower end of the "embryo sac mother cell." Of the three cells thus formed, the uppermost one, that is, the one toward the micropyle, becomes the one-celled stage of the embryo sac. TREUB has described the same for *Loranthus sphaerocarpus*. He refers to the two lower cells as "anticlines." These authors make no reference to megaspore formation, nor is the place of the reduction division known, yet we may assume that the cells just mentioned are megaspores.

#### The development of the embryo sac

Two embryo sacs are formed in each ovary, one from each chalazal nucleus, resulting from the division of the megaspore mother cell. Since the development of the two sacs is practically the same, we need follow the history of but one. The cell which gives rise to the gametophyte may be said to become the one-celled sac, and on dividing the two-nucleate sac is formed (figs. 14, 17, 26). No traces of cell walls were observed between these two nuclei. Their division results in the formation of the four-nucleate sac, in which the two nuclei at each pole of the sac are sister nuclei (fig. 27). Simultaneously with the formation of the one-nucleate sac, starch and other food materials are being laid down within its cavity, and by the completion of the four-nucleate stage it is often so densely filled with these substances that the nuclei are almost completely hidden (fig. 28). This is especially true of the sacs of *Dendrophthora gracile*. Ovules of this plant were found in which the nuclei of the sac were either degenerating or had entirely disappeared as a result of the abundant storage of food. In some examples one of the nucelli had been replaced by a cavity filled with food materials (fig. 29). The starch and other organic substances associated with it evidently serve as nourishment for the

further development of the gametophyte, and by the completion of the sac they have usually been entirely consumed. The sac toward the base of the inflorescence as a rule contains a greater amount of stored food substances than its mate, and is the one in which degeneration of the nuclei occurs. No examples were observed where the nuclei in both sacs had begun to disintegrate.

Following this stage, the end of the sac grows almost straight downward in the floral axis until it extends below the level of the insertion of the placenta. It then curves outward into the tissue of the carpel, bends, and grows upward beneath the epidermis of the inner wall of the carpel until the micropylar end of the sac lies almost over the apex of the floral axis (figs. 29, 30). At first it forms a very narrow tube, having a diameter but slightly greater than the width of the cells adjacent to it. It forces its passage through the tissues of the floral axis and the carpel by digesting the cells in front of it. The behavior of the sac suggests strongly that of a pollen tube. GRIFFITH (11), in his observations on the Santalaceae, figured and described the branching of the chalazal portion of the embryo sac of *Santalum* and mentioned that it behaved much like a pollen tube. LLOYD (18) calls attention to the pollen-tubelike behavior of the embryo sac in his studies of the Rubiaceae. The disintegration of the cells indicates clearly the secretion of an enzyme by the tubelike sac. A portion of the protoplasm in this sac seems to be specialized for this purpose, as will be shown later. As the sac advances, it evidently derives its nutriment from the cells immediately surrounding it. In a sense it is a parasite within a parasite, feeding on the tissue through which it moves. The path of growth of the sac brings it into a most advantageous position for obtaining food. It also provides a line of transit through which food is readily transferred to the developing endosperm and embryo. When the sac grows downward, it comes into direct contact with the vessels at the base of the placenta (text fig. 20). As it turns out into the carpels and grows upward, it is in close proximity to vascular traces from which it draws its nourishment. The cells of the region of the carpel through which the sac moves are richer in protoplasm than the surrounding tissue (fig. 31). No starch was found in these cells, yet their general

appearance and reaction to various stains indicate that they are supplied with some substance which serves as food for the embryo sac. Hence the direction of growth of the embryo sac may be regarded in part as a response to chemotactic stimuli. In *Loranthus sphaerocarpus*, according to TREUB, the embryo sac is surrounded by a sheath consisting of a single layer of cells which are filled with starch. During the development of the long arm from the base of the sac, the portion within the floral axis has been slowly enlarging and advancing upward. The sac as now seen is shaped like a hook, the short arm of which is within the floral axis, while the long club-shaped portion lies wholly within the tissue of the carpel.

The history of the formation of the nuclei of the sac and their arrangement within it are no less interesting to follow than the form of the sac itself. The two nuclei in the chalazal end of the four-nucleate sac become the "antipodals" of the mature sac. They do not divide, but become somewhat enlarged. They are nearly always in close contact with each other, and by the time of the origin of the embryo they may be partially fused. Still later they may become wholly fused (fig. 30), while in other cases they never unite, but remain separate until a very late period in the formation of the endosperm and embryo, when they finally disappear. Coincidentally with the downward growth of the sac, the greater portion of the cytoplasm and the two micropylar nuclei, together with most of the food substances, move into the tubelike extension as it is being formed. This mass usually lies a short distance from the apex of the sac as it works its way up through the tissue of the carpel (figs. 32, 33). The cytoplasm which is in contact with the wall of the micropylar end of the sac does not contain starch. It is very dense, finely granular, and stains more darkly than the cytoplasm of the lower portion of the sac, in which the dividing nuclei and food substances are imbedded. With iodine it stains a yellowish dark brown. It is evident that this is a specialized portion of the protoplasm of the sac which probably secretes an enzyme for digesting the tissue as it advances through the carpel. This is evident from the fact that a number of examples were found where the apex of the sac extended up between the cells of the tissue



adjacent to it in the form of pseudopodium-like projections. The cells in contact with these pseudopodia were partially digested (figs. 34, 43). The nuclei of the long arm of the sac are derived from the two micropylar nuclei of the four-nucleate stage, at which period in the development of the sac they are usually a short distance apart. Preceding their division, which begins about the time the sac commences to grow downward, they come together and lie in close contact until after they have divided. They are so intimately associated that in some examples they appear to be partially fused. In the stage preceding the upward growth of the tubelike sac, they are found partially divided in a mass near the tip of the sac, in which 6 nucleoli are distinguishable, some of which are entirely inclosed by a nuclear wall (figs. 35, 36, 38, 39). Examples were found where the sac had advanced well up into the carpel and in which the nuclei had just begun to divide (fig. 34). Either 5 or 6 nuclei are formed, and for some time after the divisions are complete they lie massed together (figs. 32, 37). About the time the tip of the sac has reached to or a little above the level of the apex of the axis, the nuclei of the sac separate, 2 occupying the position of polar nuclei, the other 3 or 4 forming the egg apparatus (figs. 41, 42, 43). The two nuclei corresponding to polar nuclei of a sac of the usual type are sister nuclei, having resulted from the division of one of the two nuclei at the micropylar end of the four-nucleate sac. From the sister nucleus of this same nucleus the nuclei of the egg apparatus are derived. There are 7 or 8 nuclei formed in each sac. If we try to homologize them with the nuclei of a sac of the usual type, we find 2 or 3 cells having the position of synergids accompanying the egg nucleus at the micropylar end of the sac, the 2 just below the egg have the position of polar nuclei, and the 2 in the chalazal region correspond to antipodals. While the long arm of the sac has been developing, the chalazal end has been enlarging and slowly advancing toward the apex of the placenta where it meets the chalazal arm of the sister sac (text fig. 20). At first they are separated by a thin wall, which finally disappears during the early stages of the formation of the embryo, so that the two sacs form one continuous tube (fig. 30).

The embryo sac of *Dendrophthora* as thus seen is quite different

from that known in other Loranthaceae. In *Viscum album*, *V. articulatum*, and *Arceuthobium Oxycedri* the sacs are similar to the common type of embryo sac. In *Loranthus sphaerocarpus* the sac is long and tubular, extending up in the narrow styler canal. In the two species of *Dendrophthora* which are the subject of this study and *Arceuthobium Oxycedri* the embryo sacs originate in a quite similar manner in the elongated floral axes, which also bear a strong resemblance in general form. The sac in the latter species grows up to the apex of the axis where it is met by the pollen tube. As already shown, there is a strong similarity between the megasporangia of *Dendrophthora*, *Thesium*, and *Santalum*. Also the sacs of *Dendrophthora* and *Santalum* are much alike in their general shape and behavior. In the latter the sac grows downward, curves, and extends upward just outside of the "mamelon." The similarity between the genera of the Santalaceae mentioned above and *Dendrophthora* in the position of the megasporangia and development of gametophytes might be taken as indicative of a phylogenetic relationship.

Of the two sacs formed in each flattened "mamelon," the one toward the apex of the spike becomes functional. The preceding description applies to this sac. It is somewhat larger and usually develops a little more rapidly than its mate. As a rule, the long arm of the latter extends a short distance above the level of the apex of the "mamelon." The development and arrangement of its nuclei are the same as in the micropylar end of the functional sac. Only a few examples of 4 nuclei in the egg apparatus were found.

The divisions of the nuclei of the sac of *Dendrophthora gracile* are usually completed much earlier than those of the sac of *D. opuntiioides*. Examples were found in which the nuclei of the long arm of the sac were formed by the time the sac had begun its downward growth (figs. 44, 45). Aside from this difference in time of division the embryo sacs, these two species of *Dendrophthora* are essentially alike. No traces of mitotic divisions of the embryo sac nuclei subsequent to that of the megaspore mother cell were observed until after the maturation of the sac. The chromatic material of the nucleus during this period appears to

have become concentrated into a single nucleolus-like body, which stains uniformly and reacts with Flemming's triple stain, Haidenhain's iron alum hematoxylin, cyanin and erythrosin, thionin, and methyl green and acid fuchsin, as chromosomes do in ordinary mitotic cell division. The division of the nuclei apparently always begins by a fission of this nucleolar mass, which is followed by a constriction of the nucleus (figs. 39, 40). This mass divides into a number of parts equal to the number of nuclei to be formed. For example, in the division of the parent nucleus of the egg apparatus, the nucleolus divides into 3 or 4 parts, each of which later becomes inclosed within a nucleus (figs. 36, 38). The staining reaction and the mode of division of the nucleolus-like body of the nucleus as thus seen clearly indicates that it is composed of chromatin, and instead of dividing into the same number of chromosomes as occur at the time of the division of the megaspore mother cell, it divides only into 3 or 4 parts. Judging from all appearances, the nuclei during the above mentioned period divide amitotically. The manner of the formation of the gametophytic nuclei is apparently quite unique, as the author has found thus far no indication in the literature that such a phenomenon has hitherto been observed in the development of an embryo sac. The mode of division of these sac nuclei is probably stimulated in some way by the presence of abundant food material within the sac.

A number of examples were found where 1-3 small bodies, which stained like the chromatic material in the nuclei of the same embryo sacs in which they occurred or as described in connection with figs. 39, 40, were present in the terminal portion of the embryo sac in the dense finely granular protoplasm mentioned above (figs. 32, 33, *y*). It was not possible to discover their origin or fate.

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#### EXPLANATION OF PLATES V AND VI

All figures, with the exception of figs. 8, 12*a*, 20, and 21, which are diagrams, are camera lucida drawings from microtome sections. Figs. 3, 6, 22, and 66 were made with a Bausch and Lomb dissecting microscope. In making the remainder of the drawings a Leitz compound microscope was used.

Abbreviations: *a*, floral apex; *an*, antipodals; *b*, bracts; *c*, swollen collar

of the inflorescence axis; *ca*, carpels; *ce*, cutinized epidermis; *cw*, wall of carpels; *d*, definitive nucleus; *dp*, degenerating polar nuclei; *e*, egg nucleus; *ea*, nuclei of egg apparatus; *em*, embryo; *en*, endosperm; *f*, young flower or floral apex; *fn*, fusion nucleus; *i*, rudimentary integuments; *m*, mamelon; *n*, nucellus; *p*, polar nuclei; *pc*, fleshy pericarp; *pl*, placenta; *pi*, pistil; *r*, remains of nuclei of egg apparatus; *s*, stem; *se*, sepal; *sn*, synergid; *st*, starch; *v*, vascular bundle; *vf*, very young flower; *x*, nuclei from which nuclei of egg apparatus and polar nuclei are derived; *y*, chromatin-like bodies.

FIGS. 1, 7, 8, 12a, 20, and 21 are text figures.

FIG. 2.—Part of longitudinal section of apex of young spike;  $\times 100$ .

FIG. 3.—Lateral view of young spike;  $\times 1.5$ .

FIG. 4.—Part of longitudinal section of apex of spike showing very young flower;  $\times 26$ .

FIG. 5.—Part of cross-section of young spike showing initiation of flower from perilem;  $\times 100$ .

FIG. 6.—Lateral view of large spike showing sequence of development of flowers;  $\times 1.5$ .

FIG. 9.—Outline of cross-section of spike showing sunken flower before sepals have begun to form;  $\times 26$ .

FIG. 10.—Outline of cross-section of spike showing sunken flowers with sepals;  $\times 26$ .

FIG. 11.—Longitudinal section of flower showing sepals and incipient carpels (*ca*);  $\times 26$ .

FIG. 12.—Longitudinal section of terminal portion of inflorescence axis showing young flowers;  $\times 10$ .

FIG. 13.—Outline of longitudinal section of flower at two-nucleate stage of embryo sac;  $\times 26$ .

FIG. 14.—Transverse section of "mamelon" at two-nucleate stage of embryo sac;  $\times 100$ .

FIG. 15.—Outline of longitudinal section perpendicular to broad surface of "mamelon" at two-nucleate stage of embryo sac;  $\times 100$ .

FIG. 16.—Section of part of "mamelon" showing megaspore mother cell with subjacent cells (*n*) which give rise to nucellar tissue;  $\times 150$ .

FIG. 17.—Longitudinal section of "mamelon" in plane of flattening, showing two-nucleate sac, nucellar tissue, and placenta;  $\times 150$ .

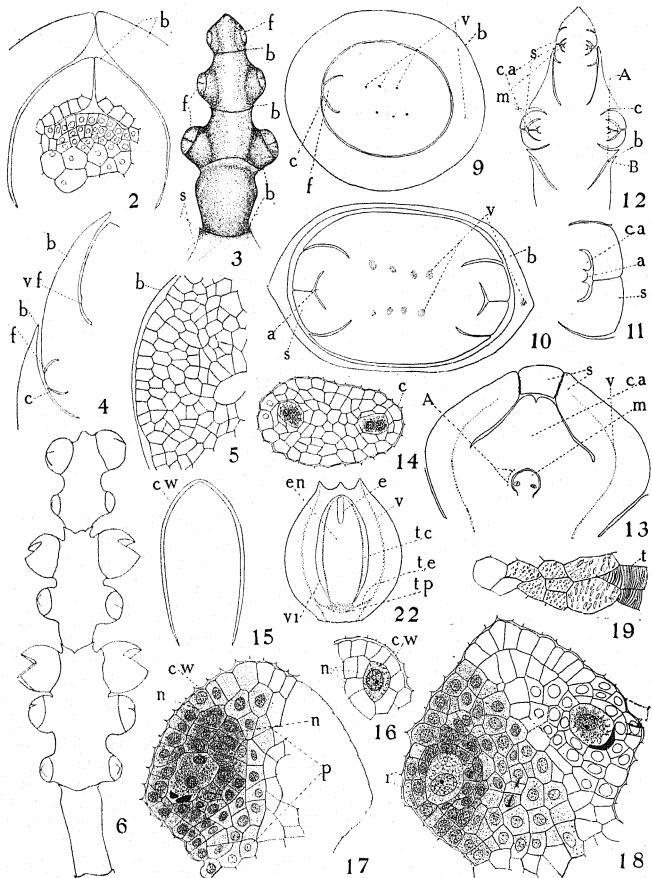
FIG. 18.—Longitudinal section of "mamelon" of *D. gracile* in plane of flattening, showing uninucleate embryo sac and rudimentary integuments (*i*);  $\times 150$ .

FIG. 19.—Portion of section of tracheid tissue at base of "mamelon";  $\times 250$ .

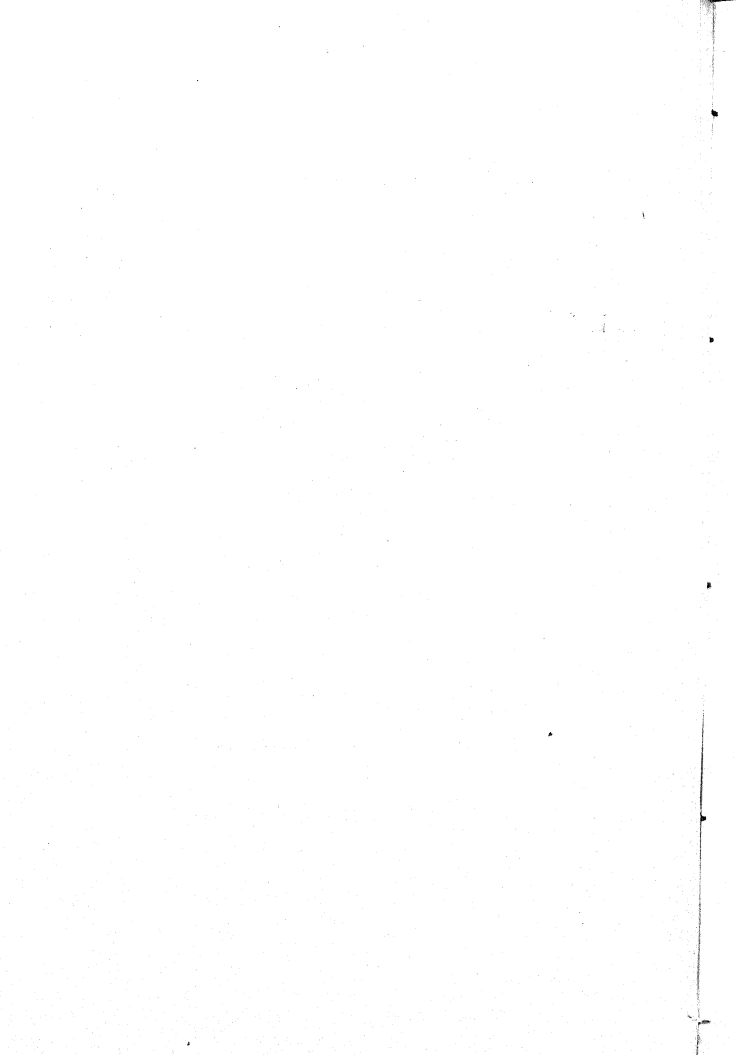
FIG. 22.—Longitudinal section of ripe berry showing embryo, endosperm, pericarp, and vascular system: *tc*, *v*, *t*, *tp*, as in fig. 21;  $\times 1.75$ .

FIG. 23.—Section of "mamelon" showing archesporial cells;  $\times 100$ .

FIG. 24.—Part of section of "mamelon," showing megaspores;  $\times 150$ .



YORK on DENDROPHTHORA



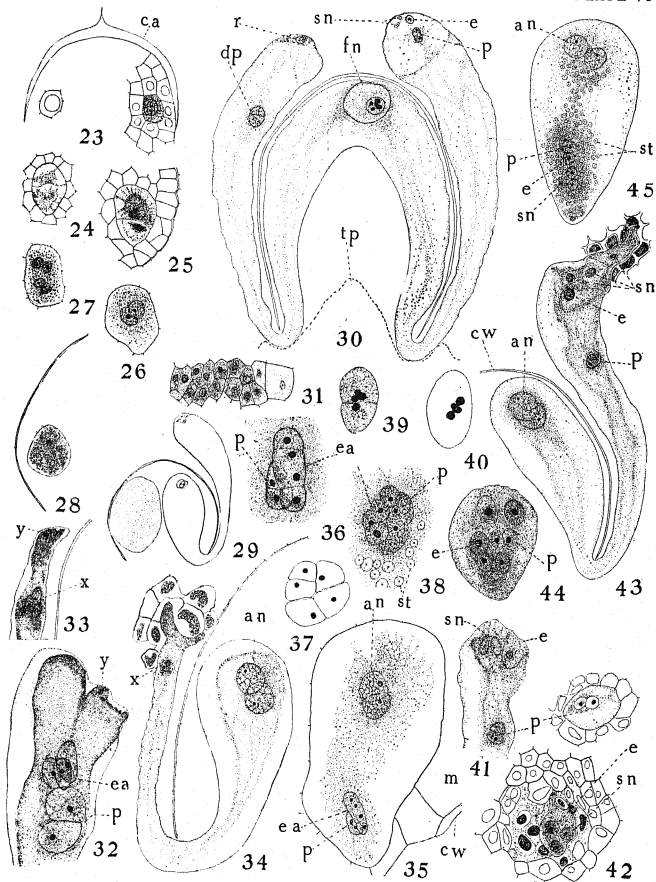






FIG. 25.—Part of section of "mamelon," showing disintegration of basal megaspore;  $\times 150$ .

FIG. 26.—Longitudinal section of two-nucleate embryo sac;  $\times 150$ .

FIG. 27.—Longitudinal section of four-nucleate sac;  $\times 150$ .

FIGS. 28, 29.—Longitudinal sections of "mamelon," showing one of the embryo sac cavities filled with starch;  $\times 100$  and 26 respectively.

FIG. 30.—Longitudinal section of flower of *D. gracile*, showing fusion of short arms of embryo sacs, resulting in formation of a single tube; *tp* same as *tp* in fig. 20;  $\times 150$ .

FIG. 31.—Part of longitudinal section of that portion of the carpel taken at *A* in fig. 13;  $\times 150$ .

FIG. 32.—Longitudinal section of upper portion of embryo sac, showing polar nuclei, nuclei of egg apparatus, and three chromatin-like bodies (*y*) in terminal portion of sac;  $\times 250$ .

FIG. 33.—Longitudinal section of upper portion of embryo sac showing chromatin-like bodies (*y*) and group of nuclei from which those of egg apparatus and polar bodies are to be derived;  $\times 250$ .

FIG. 34.—Longitudinal section of young embryo sac, showing pseudopodium-like projections;  $\times 150$ .

FIG. 35.—Longitudinal section of young embryo sac which has begun to grow downward, showing dividing nuclei;  $\times 250$ .

FIG. 36.—Detail drawing of nuclei *ea* and *p* in fig. 35;  $\times 500$ .

FIG. 37.—Nuclei at micropylar end of sister sac to the sac shown in fig. 35;  $\times 500$ .

FIG. 38.—Dividing nuclei at micropylar end of sac from which the synergids, egg, and polar nuclei are to be derived;  $\times 500$ .

FIGS. 39, 40.—Dividing nuclei showing segmentation of the nucleoli;  $\times 500$ .

FIG. 41.—Longitudinal section of terminal portion of embryo sac;  $\times 500$ .

FIG. 42.—Cross-sections of mature embryo sac of *D. gracile*;  $\times 150$ .

FIG. 43.—Longitudinal section of mature embryo sac, showing three synergids, egg, and degenerating polar nuclei;  $\times 150$ .

FIGS. 44, 45.—Longitudinal section of embryo sacs of *D. gracile* that have just begun to go downward;  $\times 250$ .

# THE PHYSIOLOGY OF THE POLLEN OF TRIFOLIUM PRATENSE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 175

J. N. MARTIN

(WITH ONE FIGURE)

The investigation of red clover pollen was begun with the hope that a thorough knowledge of its physiology, in conjunction with the history of the embryo sac, might help to overcome the uncertainty of clover seed production. The investigation was started at the University of Chicago during the summer term of 1912 and continued at Ames during the autumn of the same year. Many points demand further investigation, but the author thought it well to publish at this time, since the work cannot be resumed until the next growing season. The work has to do with three questions: conditions necessary for the germination of pollen; the stigma as a stimulative and directive factor in tube development; and relative potency of the pollen in self and cross-pollination.

## Historical

HANSGIRG (8) and LIDFORSS (9, 11) succeeded in germinating the pollen of many species in tap water or moist air. The pollen of *Trifolium hybridum* germinated in moist air, but the pollen of *T. pratense* burst. RITTINGHAUS (5) found that the pollen of a large number of species would germinate in cane sugar solutions. The optimum concentrations for the different species varied from 20 to 40 per cent. MAX PFUNDT (14) showed that 20 to 50 per cent concentrations were required for the pollen of some grasses. KNY (3) found that the pollen of *Aesculus Hippocastanum*, *Lilium bulbiferum*, *Robinia Pseudo-Acacia*, *Lathyrus tuberosus*, and *Pisum sativum* germinated better when gelatin was added to the cane sugar solution. MANGIN (4) increased the germination in some species by adding either agar or gelatin to the sugar medium. JOST (12) found the germination of the pollen of some species of grasses to depend entirely upon the water supply. This he

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controlled by germinating the pollen on parchment paper soaked in distilled water and properly dried on filter paper. The pollen of some composites would germinate on the parchment paper only after it had been soaked in sugar solution and then suitably dried. ELFVING (2) was unable to germinate the pollen of *Ornithogalum Ecklonii* and some species of grasses except on the stigma. Glycerin, potassium chlorate, and sodium carbonate added to sugar solutions had no effect. HANS MOLISCH (7) found that 0.01–0.05 per cent calcium malate or 0.01 per cent malic acid added to the sugar solution would cause the pollen of *Azalea* and *Rhododendron* to germinate. Saltpeter, asparagin, citric acid, and tartaric acid had a slight stimulative effect. LIDFORSS (9) increased the percentage of germination in some species of *Erica* and *Menziesia* by the addition of a small percentage of citric acid. VAN TIEGHEM (1) obtained better germination in some species by adding ammonium bitartrate to the medium. SANDSTEN (16) found that tomato pollen required a slightly acid medium. BURCK (12) observed that the pollen of certain species of *Mussaenda* would germinate in distilled water, but only when a portion of the stigma or levulose was added. Levulose could not be replaced by other sugars. TISCHLER (15) was able to germinate the sterile pollen common in *Solanum rostratum*, in some of the Commelinaceae, Melastomaceae, Pontederiaceae, Liliaceae, Lythraceae, and in the genus *Cassia* of the Leguminosae, by adding diastase to the sugar solution. According to LIDFORSS (9), the presence of a small percentage of calcium or potassium salts or a lack of aeration will prevent germination in many species. BURCK (12) found that levulose inhibited germination in *Pavetta* and *Antirrhinum* and caused bursting in *Murraya exotica*. The work of MOLISCH (6, 7) showed that the direction of pollen tubes in some species is due to carbohydrates, and in other species to negative aerotropism. LIDFORSS (10) found that proteins attract the pollen tubes in some species. In the species investigated by KNY (3) gravity and light had no directive influence on the pollen tubes. Some of the earlier botanists, and more recently LINDHARD (17), and the work carried on by PAMMEL and COE, which is not yet published, have shown that the pollen of *Trifolium pratense* is impotent on its own stigma.

## Description of the pollen

The pollen grains mature very early in the history of the flower, the stamens and pistil being about 0.25 mm. in length and the integuments barely appearing on the ovules when the mother cells divide. When the embryo sac is mature the pollen grains are binucleate. They are almost globular when turgid, with a little flattening around the germ pores, and have an average size of  $44.5 \times 43 \mu$ . The pollen is plasmolyzed when shed from the anther, and one diameter is much shortened by an infolding of the wall. The average dimensions in this condition are  $26 \times 48 \mu$ .

CONTENT.—Pollen treated with chloral hydrate and iodized potassium iodide gave no starch reaction. The immediate bursting of the pollen in these solutions permits good exposure of the contents and makes observations easy. Treated with Sudan III, the numerous small particles giving the pollen content its granular appearance gave a definite fat reaction. This fat exists in the form of a fine emulsion.

OSMOTIC PRESSURE.—In determining the osmotic pressure, sucrose solutions were used, since the pollen seems less permeable to this sugar. Volume-normal (mol. wt. dissolved to a liter volume of solution) solutions were used in watch glasses, which were sealed to prevent evaporation and left on the laboratory table. The results are given in table I.

TABLE I

Time	1.5 volume-normal	2 volume-normal	2.33 volume-normal	2.5 volume-normal	2.66 volume-normal
10 m. . . .	Turgid	Turgid	Plasmolyzed	Plasmolyzed	Plasmolyzed
20 m. . . .	"	"	"	"	"
30 m. . . .	"	"	"	"	"
1 hr. . . .	"	"	"	"	"
7 hrs. . .	"	"	5 per cent turgid	"	"
20 hrs. . .	"	"	10 per cent "	"	"
48 hrs. . .	"	"	20 per cent "	"	"

The table shows that a 2.33 volume-normal solution is a little weak, provided the pollen grains are not permeable to the solution; but 2.33 volume-normal is nearer the proper strength than 2.5. If 2.33 volume-normal is changed to weight-normal (mol. wt.

dissolved in a liter of water) and calculated for 25° C. according to data and formulae given by RENNER (18), it gives an osmotic pressure of 163.5 atmospheres. This takes no account of the excessive increase of pressure over concentration between 4.13 and 4.65 weight-normal (2.33 volume-normal). The pollen was permeable to saturated solutions of KNO<sub>3</sub> and NaCl, and these salts could not be used for determining osmotic pressure.

### Germination of pollen

The pollen of *Trifolium pratense* bursts almost instantly when dropped into water, so any suitable medium must control water absorption. Small amounts of sugar solutions made up in double distilled water by the volume-normal method were used in the ordinary watch glasses, carefully cleansed. The flowers were collected between 9 A.M. and 3 P.M. and the pollen from those well open, but still fresh, was used. The dishes, sealed with glass plates and vaseline, were left on the laboratory table and observations were made about every 30 minutes during the three or four-hour test period. Table II shows the effects of different sugar solutions on the pollen of *Trifolium pratense*, *T. hybridum*, and *T. repens*.

Decoctions of the stigmas alone or in distilled water, as well as those made by grinding the stigmas in the sucrose and levulose solutions given in table II, gave no germination in *Trifolium pratense*; 0.000075, 0.000375, and 0.075 volume-normal solutions of malic acid, as well as equal concentrations of calcium malate, added to the sugar solutions gave increased bursting in *T. pratense* and reduced the percentage of germination in *T. hybridum* and *T. repens*. A 0.000277 volume-normal of HCl or a 0.00056 volume-normal of butyric acid used with the sugar solutions had little effect. The butyric acid gave a little better germination in *T. hybridum* and *T. repens* in sucrose solutions above 0.731 volume-normal. Stronger solutions of either acid increased bursting and cut down germination. Sugar solutions containing agar or gelatin allowed less bursting, and 2 grams to 5 grams of gelatin added to a 0.731 volume-normal solution of sucrose gave the best medium for the pollen of *T. hybridum* and *T. repens*. Pollen of *T. pratense* run in sugar solutions under increased pressure of oxygen and

TABLE II

SHOWING THE EFFECTS OF DIFFERENT SUGAR SOLUTIONS ON THE POLLEN OF *Trifolium pratense*, *T. hybridum*, AND *T. repens*

Solution	Volume-normal	<i>T. pratense</i>	<i>T. hybridum</i>	<i>T. repens</i>
Double distilled H <sub>2</sub> O.....		Immediate bursting	Immediate bursting	Immediate bursting
Sucrose ...	0.1462	Bursting	Bursting	Bursting
" ...	0.2824	"	"	"
" ...	0.4386	"	Bursting 25; germination	Bursting 25; germination
" ...	0.5848	About 50 burst; no germination	About 80 per cent germination	About 80 per cent germination
" ...	0.731	Turgid	Good germination	Good germination
" ...	0.8772	"	" "	" "
" ...	1.0233	"	" "	" "
" ...	1.1695	"	Plasmolysis	Plasmolysis
" ...	1.3217	"	"	"
" ...	1.4619	"	"	"
Levulose ...	0.833	Bursting	Some germination and much bursting	Some germination and much bursting
" ...	1.1108	About 50 per cent bursting	Fair germination; no bursting	Fair germination; no bursting
" ...	1.3888	Turgid; no bursting	About 50 per cent germination; no bursting	About 50 per cent germination; no bursting
" ...	1.666	Turgid; no bursting	About 50 per cent germination; no bursting	About 50 per cent germination; no bursting
" ...	2.2216			
" ...	2.499			
" ...	2.7776			
Dextrose ...	1.1108	Bursting	Bursting	Bursting
" ...	1.3888	"	Feeble germination; some bursting	Feeble germination; some bursting
" ...	1.666	"	Feeble germination; some bursting	Feeble germination; some bursting
" ...	1.9333	"	No bursting; 25 per cent germination	No bursting; 25 per cent germination
" ...	2.2216	"	No bursting; 25 per cent germination	No bursting; 25 per cent germination
" ...	2.499	"	No bursting; 25 per cent germination	No bursting; 25 per cent germination
" ...	2.7776	"	Bursting	Bursting
Maltose ...	0.5848	Some bursting	Feeble germination; some bursting	Feeble germination; some bursting
" ...	0.731		Feeble germination; some bursting	Feeble germination; some bursting
" ...	0.8772	Bursting	Feeble germination; some bursting	Feeble germination; some bursting
" ...	1.0233	"	No bursting; 25 per cent germination	No bursting; 25 per cent germination

TABLE II—Continued

Solution	Volume-normal	<i>T. pratense</i>	<i>T. hybridum</i>	<i>T. repens</i>
Maltose...	1.1695 1.3217 1.4619	Bursting	No bursting; 25 per cent germination	No bursting; 25 per cent germination
Percentages of maltose and dextrose mixed gave about the same results				
Lactose...	0.5848	Bursting	Bursting	Bursting
" ...	0.731	"	50 per cent germination; some bursting	50 per cent germination; some bursting
" ...	0.8772	"	50 per cent germination; some bursting	50 per cent germination; some bursting
" ...	1.0233 1.1695 1.3217 1.4619	"	50 per cent germination; no bursting	50 per cent germination; no bursting
Arabinose	1.3333	"	Bursting	Bursting
"	1.6555	"	50 per cent germination; some bursting	50 per cent germination; some bursting
"	1.9999	"	50 per cent germination; no bursting	50 per cent germination; no bursting

carbon dioxide showed that the supply of these gases was not the limiting factor. Small dishes made by cutting off glass shells about one-half inch from the bottom and containing the pollen in very shallow depths of sucrose and levulose solutions, which did not permit bursting, were placed in wide-mouthed bottles and attached to oxygen and carbon dioxide tanks. No germination resulted from a three-hour exposure to an increased pressure of these gases. In table III the results obtained with other media are given.

Results obtained by use of parchment paper and animal membrane with pollen of *Trifolium pratense* and  
*T. hybridum*

Small squares of parchment paper were soaked in distilled water and in 0.5848, 0.731, and 0.8772 volume-normal sucrose solutions and then dried on filter paper until surplus moisture was removed, mounted on slides, and after application of pollen placed

under bell jars on the laboratory table. Parchment paper proved unsatisfactory because its opaqueness and fibrous character made observation difficult; so after several sets were run with no germination, hog bladder was substituted. After a few trials good

TABLE III

SHOWING THE EFFECTS OF VARIOUS SOLUTIONS ON THE POLLEN OF *Trifolium pratense*

Solutions			
Sucrose 0.731+KNO <sub>3</sub> 0.00006.	Bursting		
Sucrose 0.731+KNO <sub>3</sub> 0.0002..	About 25 per cent bursting	75 per cent turgid	No germination
Sucrose 0.731+KNO <sub>3</sub> 0.0006..	About 25 per cent bursting	75 per cent turgid	" "
Sucrose 0.731+KNO <sub>3</sub> 0.002..	Very little bursting	Turgid	" "
Sucrose 0.731+KNO <sub>3</sub> 0.005...	No bursting	"	" "
Sucrose 0.731+galactose 1.3888 + a trace of asparagin.....	Very little bursting	"	" "
Sucrose 0.731+asparagin 0.0375.....	Bursting		
Sucrose 0.731+dextrose 1.3888 +KNO <sub>3</sub> 0.0005.....	Little bursting	25 per cent or more turgid	" "
Sucrose 0.731+0.0075 asparagin.....	About 50 per cent bursting	50 per cent turgid	" "
Sucrose 0.8772+dextrose 1.666 +asparagin 0.075.....	Bursting	.....	.....
Sucrose 1.0233+asparagin 0.15	"	.....	.....
Sucrose 0.8772+galactose 1.9333+asparagin 0.187....	"	.....	.....
Sucrose 0.8772+asparagin 0.0015.....	No bursting	Turgid	" "
Sucrose 0.8772+asparagin 0.00075.....	" "	"	" "
Sucrose 0.8772+asparagin 0.000375.....	" "	"	" "
Sucrose 0.8772+glycerin oleic acid.....	" "	"	" "
Sucrose 0.8772+palmitic acid..	" "	"	" "
Sucrose 0.8772+lipase.....	" "	"	" "
Levulose 1.666+lipase.....	" "	"	" "
Lecithin+H <sub>2</sub> O as a thick paste.	" "	"	" "
Sucrose+lecithin as a thick paste.....	" "	"	" "
Levulose+lecithin as a thick paste.....	" "	"	" "
Sucrose 0.8772+diastase.....	" "	"	" "

germination was obtained on the bladder. It was found that germination was very closely connected with the amount of water in the membrane, and it was not easy to dry the membrane so as always to secure germination. Membranes soaked in



0.731 and 0.8772 volume-normal solutions of sucrose or in 1.3888 and 1.666 volume-normal solutions of levulose and properly dried gave as good results as those soaked in distilled water. This shows that these sugars have no toxic effect on the pollen. The efficiency of the membrane did not depend upon the fats or salts contained, for pieces extracted 5 days in alcohol and ether in a Soxhlet's extraction apparatus or boiled for 16 hours in changes of distilled water did not lose their efficiency, although their physical properties were so changed that the requisite amount of soaking and drying had to be found again by experimentation. Another series of trials with parchment paper showed it to be as effective as the bladder and that the previous failure was due to insufficient drying. In these tests most attention was given to the pollen of *T. pratense*, although the pollen of *T. hybridum* was investigated sufficiently to discover that it germinated readily on the membrane and that its germination would permit more variation in the water content of the membrane than the pollen of *T. pratense*.

The nature of the germination of the pollen of *T. pratense* on the membrane needs some discussion. Germination was not uniform. On some portions of the membrane the percentage of germination was high, while on other portions there was no germination. These different regions were usually quite definitely marked off, and the germination in a region was usually good or none at all. This lack of uniformity was mainly due to a difference of texture, composition, or thickness of these regions. A difference in these properties would make a difference in the amount of water supplied to the pollen in the different regions. Some variation no doubt exists between pollen from different anthers and between pollen grains from the same anther in respect to the water supply requisite for germination. But in mounting the pollen, the keel was sprung with a scalpel, and as the pollen was thrown from the anthers, it was collected on the instrument and then spread on the membrane. By this method of collecting and mounting, the pollen was well mixed, and the variation of the pollen would not account for all of the lack of uniformity in germination.

The percentage of germination determined by taking into account all the pollen on a membrane when any germination occurred

varied from almost zero to 96 on different membranes. Still, on membranes with the low percentage of germination, the percentage of germination was high in the region where it occurred. In table IV are given the results obtained on a small piece of bladder that gave fairly uniform germination.

TABLE IV

Total number of pollen grains	Number of germinations	Percentage of germination
107.....	87	81+
135.....	110	81+
209.....	183	87+
176.....	170	96+
126.....	101	80+
153.....	144	94+

The time required for germination at room temperature was 8-10 minutes. This agrees with SANDSTEN'S (16) report on *T. hybridum* and *T. repens*.

The lengths of tubes produced were various. The maximum length of tubes measured was about 15 times the diameter of the pollen grain. The lengths of the majority ranged from 6 to 15 times the diameter of the pollen. It is probable that much longer tubes would have been produced if the water delivery of the membrane had remained constant.

The results obtained with the membrane and parchment paper showed that the water supply was at least the important factor if not the only factor in determining germination.

An attempt was made to secure the proper water supply by means of sugar solutions. Sucrose solutions with a difference of 0.0877 volume-normal and ranging from 0.731 to 2.2 volume-normal were used. The only germination obtained in these solutions was less than 0.5 per cent in 1.7 volume-normal. In accounting for this failure, three things should be considered: (1) the range of water supply permitting germination may be so small that it was missed by these concentrations; (2) the supply of oxygen and carbon dioxide might have been limiting factors since the higher concentrations were greater than those run under the increased pressure of these gases; (3) the condition for germina-

tion may be a certain ratio between the amount of water taken up and transpiration.

An effort was made to reduce this required water supply to some definite expression by running tests on bladder suspended over different concentrations of  $H_2SO_4$ . Gram-molecular solutions were placed in large, wide-mouthed bottles fitted with rubber corks. A glass tube about 10 inches in length was run through the rubber cork and the membrane suspended from a cork fitted over the lower end of the glass tube. With the upper end of the tube corked, the apparatus was left in the required temperature 48 hours to secure moisture equilibrium between liquid and membrane. If stored longer than 48 hours fungi gave trouble. The pollen was collected on the end of a glass rod and deposited on the membrane by running the rod through the glass tube. This method prevented interchange between outside and inclosed air. The percentages of moisture were approximated from data given in LANDOLT-BÖRNSTEIN (19). The humidity at the pressure of saturation over pure water was considered 100 per cent, and moisture for each temperature used and the percentages over the solutions are based on the 100 per cent. The results of two series run are given in full to show variation and the others are summarized (tables V-VII).

TABLE V  
TEMPERATURE 35° C.

Gram molecular of $H_2SO_4$	Relative percent. of moisture	No. of pollen grains	Percent. of germinations	Condition
1.....	95.5	1201	0.88	Turgid
0.7.....	96.5	2187	17.4	Some bursting
0.5.....	97.2	1801	29.9	Little bursting
0.3.....	98.6	2209	10.0	20 per cent bursting
Pure $H_2O$ .....	Some less than 100	2736	24.4	30 per cent bursting

TEMPERATURE 20° C.

1.....	95.5	1120	0	Turgid
0.7.....	96.5	1396	0.21	"
0.5.....	97.2	1590	0.12	"
0.3.....	98.6	1340	1.34	Little bursting

TABLE VI

RELATIVE PERCENTAGE OF MOISTURE 98; GRAM-MOL. 0.5  $H_2SO_4$ ; TEMPERATURE  $35^\circ C$ .

No. of sets run	No. of pollen grains	No. of germinations	Percentage of germinations	Condition
1st.....	208	0	0	Turgid
2d.....	200	0	0	"
3d.....	198	0	0	"
4th.....	205	125	60.9	"
5th.....	223	146	64.5	Some bursting
6th.....	181	5	2.7	Turgid
7th.....	208	0	0	"
8th.....	195	165	84.6	"
9th.....	183	98	53.5	"
Totals.....	1801	539	29.9	

TABLE VII

RELATIVE PERCENT. OF MOISTURE 99; GRAM-MOL. 0.3  $H_2SO_4$ ; TEMPERATURE  $35^\circ C$ .

No. of sets run	No. of pollen grains	No. of germinations	Percentage of germinations	Condition
1st.....	219	0	0	Turgid
2d.....	167	0	0	50 per cent bursting
3d.....	127	0	0	Turgid
4th.....	132	0	0	"
5th.....	253	50	19.7	"
6th.....	218	100	45.8	"
7th.....	310	10	3.2	Much bursting
8th.....	435	35	8+	" "
9th.....	348	26	7.5	" "
Totals.....	2209	221	10	

As seen from the tables, the percentages of germination in most of the sets run were low as compared with those obtained under bell jar on the laboratory table. This low percentage may be due to three things: (1) the membrane was not in equilibrium; (2) the amount of moisture required by different pollen grains for germination may vary so much that only a small percentage of germination can take place under a given moisture condition; (3) germination may be to some extent connected with transpiration. The marked variation in behavior between sets run over the same solution at the same temperature strongly suggests that moisture equilibrium had not been established within the apparatus. The

tables show that for these temperatures germination takes place only when the percentage of moisture is close to saturation.

**The influence of the stigma upon the germination of the pollen  
and upon the direction of the pollen tubes**

The stigma presents a very uneven surface due to the projection of the papillae. The exposed portion of the papillae has a rather heavy cutinized wall. Microchemical tests showed no sugar or starches present in the papillae, but an oily emulsion such as was found in the pollen.

Although decoctions of the stigmas had been tried without any positive results, it was thought worth while to try them in connection with the bladder. After the pollen had been spread on the prepared sections of bladder, stigmas from other plants were pressed down on these membranes with a scalpel, and then the sets were run under the bell jar on the laboratory table. These stigmas apparently exerted no influence on germination or on the direction of the pollen tubes. Often there was no germination around the stigma when there was good germination in other regions; and when there was germination around the stigma, germination just as good could be found in other places. The pollen tubes around the stigmas were grown in all directions, and pollen grains in contact with the stigma were found growing tubes at right angles to, and away from, the stigma. From these observations it appears that the stigma secretes nothing that has any effect on germination or the direction of the pollen tube. The behavior of the stigma in the experiments at least indicates that its function in the germination of the pollen is to regulate the water supply; and the nature of the pollen necessitates no other function. If this is the function of the stigma, and the water supply must be as delicately adjusted on the stigma as on the membrane to secure germination, then conditions which will modify the amount of water delivered by the stigma will have an effect on fertilization and hence on seed production. This may account for the usually poor seed production in the early part of the season, since there is usually more moisture in the ground at this time and more rain during the flowering period than occurs during the second crop. If germination depends

upon a certain balance between the amount of water taken up and transpiration, then a variation in the moisture of the atmosphere would have an effect on fertilization.

### The comparative potency of pollen in self and cross-pollination

Pollen tubes can be traced through the stylar canal by mounting the pistils in a 30 per cent sucrose solution and flattening with

a little pressure on the cover glass. The tubes have a denser and more granular content than the cells of the style and these features make it possible to trace them. Sufficient pressure causes the ovary to break just above the ovules and enables one to see the tubes in the ovary.

Fig. 1 shows the upper portion of an ovary with exposed pollen tubes 55 hours after pollination. For self and cross-pollination vigorous field plants were selected, and flowers to be used were put under cover 2 or 3 days previous to opening and remained covered until collected for examination.

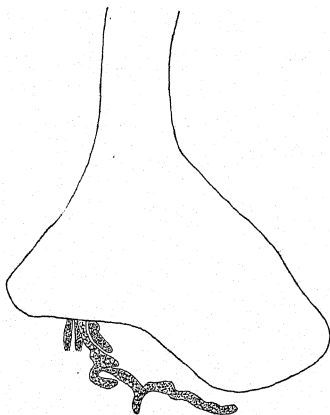


FIG. 1.—Camera drawing, showing the upper portion of an ovary teased off and the pollen tubes invading the ovary.

nation. Flowers were self-pollinated by simply springing the keel. In cross-pollination the keel was sprung, and the pollen collected from another plant was applied to the stigma with a scalpel. An examination of 30 flowers crossed showed the pollen tubes in the ovary 50 hours after pollination. Sections of ovaries killed 55 hours after crossing showed that the egg had enlarged for its first division

and that the endosperm cell had made one division; therefore, fertilization must take place about 50 hours after cross-pollination.

An examination of those flowers self-pollinated at the same time the others were crossed showed good germination on the stigma. The number of pollen grains germinating on their own stigma ranged from 3 to 25 in the 30 flowers. The tubes produced were all short, none exceeding 4 mm. Out of 20 self-pollinated flowers run 90 hours, one tube was found with a length of 7.25 mm.; the other tubes varied in length from a fraction of a millimeter up to 5 mm. Counting the average length of the style and stigma 11.5 mm., one is able to compare the rates of growth of pollen tubes in the two cases. The tubes in case of self-pollination look as vigorous as those in cross-pollination. Some abnormal behavior was observed. In one case the tubes were found wound about each other in the upper part of the stylar canal. In a few cases one of the longer tubes had turned back upon itself.

The question is now raised in case of self-pollination as to whether or not the tube can reach the ovary and effect fertilization. Field work on self-pollination shows that it rarely does, if ever.

### Summary

The pollen of *Trifolium pratense* is physiologically different from that of *T. hybridum* and *T. repens* in respect to behavior toward sugar solutions.

The only function of the sugar solution in the case of the pollen of *T. hybridum* is the controlling of water supply.

The germination of the pollen of *T. pratense* is delicately adjusted to water absorption.

The results of the investigation show that the stigma produces no secretions which influence pollen tubes.

The nature of the pollen demands no other function of the stigma in its germination than the control of the water supply.

The pollen in self-pollination germinates readily on the stigma, but the tubes traverse the style much more slowly than in cross-pollination.

In conclusion I wish to express my thanks to Dr. WILLIAM CROCKER and Dr. SOPHIA ECKERSON of the University of Chicago,

under whom a greater part of the work was done, for their valuable suggestions; and to Dr. PAMMEL, who gave me valuable assistance at Iowa State College.

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## OBSERVATIONS ON THE MORPHOLOGY OF THE AROIDS

JAMES ELLIS GOW

(WITH FORTY-SEVEN FIGURES)

The material for the following observations was collected in the greenhouses of the New York Botanical Gardens, Bronx Park, New York, and was worked up by the writer during the years 1911 and 1912. The usual methods were used, the sections being cut in paraffin, and stained usually by the iron-alum-hematoxylin process, or triple stained with Delafield's hematoxylin, safranin, and orange G. The species are given in the order studied:

### 1. *Aglaonema commutatum*

At the time this work was begun, the writer was not aware of the work done by CAMPBELL on this species.<sup>1</sup> The results confirm CAMPBELL's conclusions as to the seven antipodals (fig. 1) and the spherical proembryo (fig. 2). In the specimen here figured, two of the antipodals appear in process of disintegration.

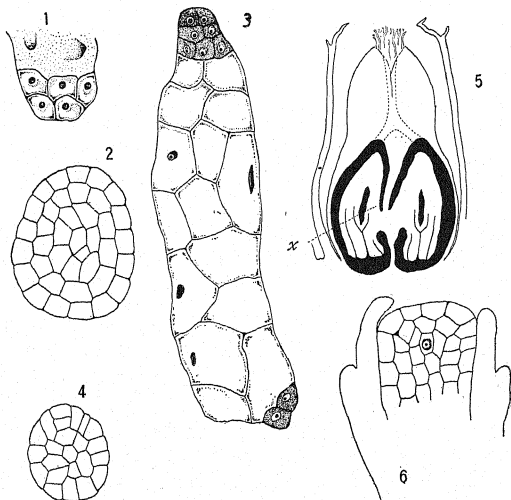
### 2. *Aglaonema nitidum*

The staminate flower consists of a synandrium, cleft into lobes by 2-5 shallow creases, each lobe containing a single loculus. The youngest material examined showed the tapetum largely broken down, the pollen grains fully formed, and the first nuclear division completed. The division of the generative nucleus had not taken place. It probably occurs in the tube, and not in the pollen grain.

The pistillate flower consists of a single carpel which contains one basally attached anatropous ovule. The inner integuments are massive, the outer being thinner and never closing over the inner. In the mature embryo sac there are 10 cells, 5 of which occupy the position of antipodals, the other 5 performing the usual functions. The divisions of the endosperm nucleus give rise to a quantity of heavy-walled endosperm (fig. 3). The antipodals, or

<sup>1</sup> CAMPBELL, D. H., Studies on the Araceae. Ann. Botany 14:1-25. pls. 1-3. 1900.

some of them, frequently persist until the sac is quite filled with endosperm. The embryo develops simultaneously with the endosperm, and is at first of the spherical form which appears to be characteristic of this family (fig. 4).



FIGS. 1, 2.—*Aglaonema commutatum*: fig. 1, seven antipodals, two disintegrating; fig. 2, spherical proembryo.

FIGS. 3, 4.—*Aglaonema nitidum*: fig. 3, endosperm; fig. 4, proembryo.

FIGS. 5, 6.—*Anthurium crystallinum*: fig. 5, longitudinal section of ovary, showing two of the six ovules; fig. 6, primary archesporial cell.

### 3. *Anthurium crystallinum*

The flowers are perfect, the number of stamens varying from 4 to 6. There is a broad connective, and complete differentiation of filament and anther, in which respect the plant differs from such forms as *Arisaema* and *Aglaonema*. The appearance of the tetrads

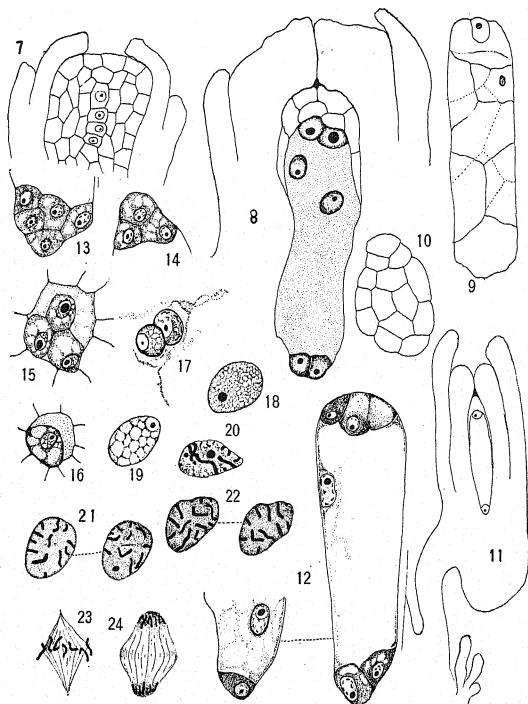
indicates that the divisions are simultaneous. The separation of the two male nuclei follows immediately on the liberation of the pollen grain in the loculus.

The compound ovary consists sometimes of two but usually of three closely united carpels, with a single truncate style whose canal is filled with viscid conducting tissue. Each of the three carpellary cavities contains two laterally attached anatropous ovules (fig. 5). The immature ovules are at first erect, but soon become reversed through the non-development of their lower surfaces. Simultaneously with their reversal, the primary archesporial cell becomes differentiated, and can readily be detected by its heavy chromatin network and ready reaction to hematoxylin stain (fig. 6). Two divisions occur, giving rise directly to 4 megaspores (fig. 7), the lower 3 of which are suppressed and break down. The functioning megaspore increases rapidly in size. The nuclear divisions are of the usual type, resulting in the formation of 8 cells. Egg, synergids, and antipodals are well marked by their positions (fig. 8). After fertilization the endosperm develops rapidly, and soon fills the embryo sac with a mass of firm, heavy-walled tissue (fig. 9), after which the division of the fertilized egg produces the usual form of proembryo (fig. 10). The ultimate fate of the endosperm in this species is not known.

#### 4. *Philodendron Wendlandii*

The staminate flowers crowd the upper part of the slender spadix and consist each of a single, 4-loculate stamen without floral envelopes. Dehiscence is along longitudinal grooves. The divisions of the mother cells are simultaneous, producing the usual type of tetrad. The tapetum is rather thin (3-4 layers of cells) and breaks down early, the nuclei sometimes floating free for a time before complete disintegration takes place.

Nine carpels are arranged radially around a common axis, forming a single compound pistil. Each carpel contains two slender pseudo-anatropous ovules. It is probably correct in this case to regard the placenta as the suppressed floral axis and the ovules as lateral shoots (fig. 11). The thin integuments project far over the tip of the nucellus, the lateral portion of which becomes sup-



FIGS. 7-10.—*Anthurium crystallinum*: fig. 7, four megaspores, the uppermost one of which functions; fig. 8, embryo sac, showing six of the eight cells; fig. 9, embryo sac filled with endosperm, the latter partially diagrammatic; fig. 10, egg-shaped proembryo.

FIGS. 11, 12.—*Philodendron Wendlandii*: fig. 11, ovule of peculiar reversed type found in this and a few other species; fig. 12, embryo sac with contents.

FIGS. 13-24.—*Xanthosoma* sp.: fig. 13, the 5 antipodals; fig. 14, egg apparatus; fig. 15, same in diagonal section of embryo sac; fig. 16, transverse section showing egg; fig. 17, polar nuclei about to fuse; fig. 18, nucleus of somatic cell in early prophase; figs. 19, 20, later prophase; figs. 21, 22, nuclei showing 16 chromosomes; figs. 23, 24, spindle.

pressed through the pressure of the growing embryo sac. The latter is 8-celled, the arrangement being of the usual type (fig. 12).

The endosperm is heavy-walled as in *Anthurium*, and the proembryo is egg-shaped.

#### 5. *Philodendron gloriosum*

The ovule is much like that of the species just described. Like it the embryo sac is 8-celled, the endosperm is heavy-walled, and the embryo spherical to ovate.

#### 6. *Arum maculatum*

This plant was obtained from a local florist and grown in pots. Owing to difficulty in the fixation of material the earlier phases cannot be given. It has the usual 8-celled embryo sac and spherical proembryo, and a thin-walled endosperm which is persistent in the seed.

#### 7. *Xanthosoma* sp.

Through accident the label attached to this specimen was lost in transit. As the material was cut into cubes and in the fixing medium its identity could not be traced.

Each cell of the tricarpellate ovary contains two slender anatropous ovules. A series of long conducting cells lines the interior of each carpel and extends to the micropylar extremity of the ovule. The extremity of the truncate style is covered with glandular papillae. The ovule is extremely slender, as in *Philodendron*, but is sessile and does not occupy the reversed position. There appear to be 5 antipodals (fig. 13), the other contents of the embryo sac being what might be anticipated in any angiosperm (figs. 14-17).

Many of the cells surrounding the embryo sac were found to be in an active state of division, probably owing to the increase necessitated in the accessory tissue by the enlargement of the embryo sac. This gave a good opportunity to study the phases of homotypic division. No continuous spirem stage could be discovered. The chromatin reticulum is at first very fine, the strands later shortening and thickening, and finally breaking into an irregular mass of coarse threads which soon resolve themselves definitely into 16 chromosomes. Early splitting of the chromosomes was noticed in a few cases (figs. 18-24).

### **Homalomena argentea**

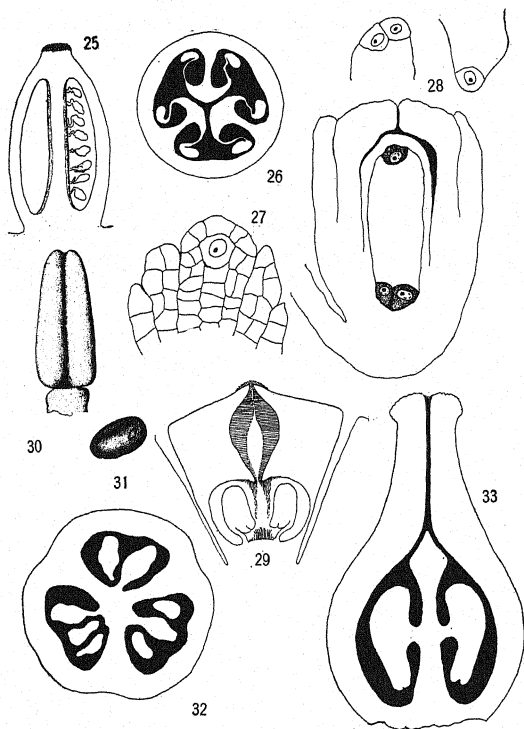
The lower part of the cylindrical spadix is crowded with the pistils, interspersed with numerous staminodia; the upper is crowded by the equally numerous stamens. Each pistillate flower consists of a single ovary, which in turn is made up of 3 foliaceous carpels. The ovules spring from the infolded edges of the placentae, and are arranged in 6 ranks (fig. 26). There is no open stylar canal, but the inner tissues of the style are loose and spongy (fig. 25). The anatropous ovule is mounted on a slender funiculus. When it has fully developed, the inner integument is massive and closes over the tip of the nucellus (fig. 28). The outer remains as shown in the figure.

The primary archesporial cell is somewhat larger than the surrounding cells (fig. 27). It divides once transversely, and of the two resultant cells the outer one functions, the inner one being broken down and absorbed. Whether the latter is to be regarded as one of a "row of two" or as a vestigial tapetum is purely an academic question. Functionally it is a tapetal cell. The embryo sac contents consist of the usual 8 cells arranged in the typical manner (fig. 28).

### **9. Stenospermation popayanense**

A curious anomalous form was found in the Botanical Gardens under the name *Stenospermation popayanense*. This may be a monstrosity, but of such a sort that its description should be of interest, and may have some morphological significance. Only one plant was found, and it bore no staminate flowers, and apparently no normal stamens, although *Stenospermation* is described by SCHOTT as having hermaphrodite flowers, with "stamina quattuor, singulo pistillo annexa." It may be, therefore, that this is not a member of the genus *Stenospermation*, although so labeled. The specific name *popayanense* is an old synonym of the original name *popayanense*.

In the form under discussion, whatever its real identity, the long slender spadix is covered with lozenge-shaped blossoms which remind one at first glance of the closed blossoms of *Symplocarpus foetidus*. The resemblance is purely superficial. In *Symplocarpus*



FIGS. 25-28.—*Homalomena argentea*: fig. 25, longitudinal section of ovary; fig. 26, transverse section of ovary; fig. 27, primary archesporial cell; fig. 28, embryo sac with six of the eight cells.

FIGS. 29-31.—*Stenospermation popayanense* (?): fig. 29, vertical section of carpel, showing ovules in position, and the stylar canal filled with conducting cells; fig. 30, stamen in cavity of pistil; fig. 31, pollen grain.

FIGS. 32, 33.—*Richardia africana*: fig. 32, transverse section of ovary, showing position of ovules; fig. 33, longitudinal section cutting two ovules.

the lozenge is formed by the infolded edges of the floral envelopes, while the central projecting "button" is produced by the protrusion of the anther tips. In the so-called *Stenospermation* the lozenge is merely the smooth upper surface of the thick-walled carpel, and there are no floral envelopes and no stamens surrounding the carpel. The "button" is the projecting stigmatic area (fig. 29). In the cavity of the carpel are found a circle of 4-8 erect anatropous ovules. Owing to the hardening of the gum contained in the tissues of this plant (and which resisted all reagents), sections could not be cut in paraffin, and the internal structure of the ovule cannot be described. Rather thick sections of the carpel were cut in celloidin, showing details of the stylar canal, and the curious crowded conducting cells as figured.

While all the blossoms are alike so far as outward appearance is concerned, a certain number of them (about 40-50 per cent) are found to bear stamens in place of the ovules just described. They are arranged in a circle, similar to the arrangement of the ovules, have large, well developed anthers with a broad connective, and when examined appeared about ready for dehiscence. The filaments of course are extremely short. If they lengthen later, no suggestion of such lengthening appeared in the material under examination. The exterior appearance of the "ovary," if such it may be called, is exactly the same whether it contains ovules or stamens. When torn open and mounted in glycerin, the anthers were seen to be filled with matured and normally formed pollen grains. No extra-carpellary stamens were found. The staminate carpels (if such they may be called) were found mixed among the pistillate, but somewhat more numerous toward the upper end of the spadix. In a very few cases the same carpel was found to house both stamens and ovules.

#### 10. *Arisaema triphyllum*

The embryogeny of this species has been already discussed.<sup>2</sup> For the investigation of the microsporangium bulbs were collected in September and the blossoms dissected out. It was found that

<sup>2</sup> GOW, JAMES ELLIS, Embryogeny of *Arisaema triphyllum*. BOT. GAZ. 45:38-44. figs. 24. 1908; also MOTTIER, DAVID M., On the development of the embryo sac of *Arisaema triphyllum*. BOT. GAZ. 17:258-260. pl. 18. 1892.



the entire microsporangial structure was fully matured at that time; the mother cells had disappeared and the pollen grains were found lying free in the loculi of the anther. Almost all the bulbs were such as had borne pistillate blossoms the preceding season; in fact the material was collected after the early frosts and was discovered by means of the conspicuous clusters of bright red berries after the withering of the leaves. A very few of the bulbs were laterals formed from the central bulb during the growing season. Two or three of the bulbs collected contained a monoecious spadix. Two-thirds of the balance contained staminate blossoms only. *Arisaema* seldom bears staminate and pistillate flowers on the same spadix, but the indication is that the plant that bears pistillate flowers one year bears staminate flowers the next. It is, in a way, consecutively monoecious, seldom simultaneously monoecious.

The loculi of the stamens contained many normal pollen grains, but far more small suppressed grains, the ratio being about 2:1, suggesting the inability of the plant to muster sufficient energy to mature all the pollen it produces.

#### 11. *Richardia africana*

This plant, the *Calla aethiopica* of the florists, was found in every stage of development at the Cedar Rapids greenhouses, and fixation proved extremely easy owing to the absence of mucilage in the tissues.

The upper portion of the slender spadix is covered by a densely crowded mass of stamens, while the pistillate flowers occur below. SCHOTT describes the pistils as "organis neutris tribus cincti," but in perhaps a fourth of the blossoms these "neutral organs" turn out to be functioning stamens, while in the remainder they are staminodia, or staminal rudiments.

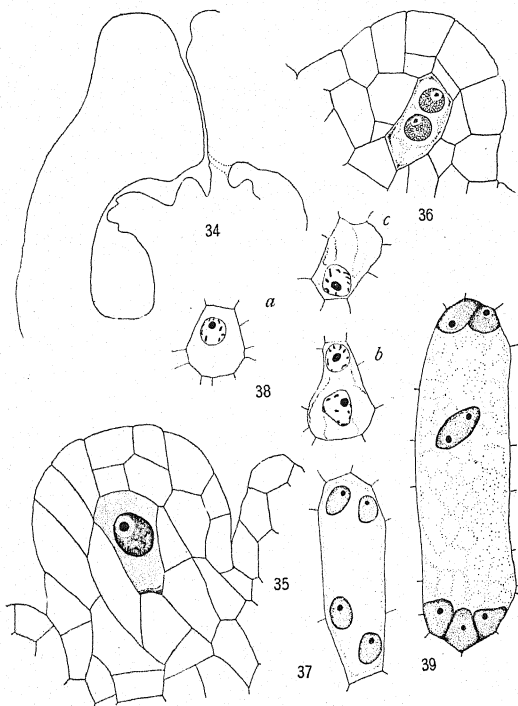
The cylindrical ovary consists of 2, 3, or 4 carpels, the anatropous ovules being borne on the central placenta, which here represents the united carpellary walls. Two to four ovules occur in each carpellary cavity (fig. 32), and a separate branch of the open stylar canal runs to each cavity. The ovule is anatropous, and of foliar origin, since it springs from the carpellary wall. The

funiculus is laterally attached. There are the usual two integuments, which grow well out beyond the tip of the nucellus (fig. 33). The ovule first appears as a lateral projection nearly filling the cavity of the immature carpel. As the latter enlarges, the ovule keeps pace with it. The integuments begin to appear before it assumes the anatropous position (fig. 34). The primary archesporial cell is differentiated about simultaneously with the appearance of the integument, and even in its resting condition may be recognized by its greater size (fig. 35). After, or simultaneously with, its differentiation, the tip cells of the nucellus divide by periclinal walls, forming two cell layers outside the archesporium. No primary parietal cell is formed. The primary archesporial cell is in this species the spore mother cell, and develops directly into an embryo sac without the previous formation of a row of megaspores. The first division occurs early (fig. 36). The later divisions follow in the usual order (figs. 37-39), and before their completion the integuments have reached complete maturity, closing over the tip of the nucellus.

The account just given represents the normal development of the ovule and embryo sac in *Richardia* and holds true in perhaps 5 per cent of the plants examined. In most cases, however, the plants not only fail to set their seed, but will not do so even when artificially pollinated. I refer here solely to the plant as I have observed it growing in local greenhouses, and to the individuals (some 150) on which I have tried the experiment of artificial pollination. The observation of gardeners, so far as they have been consulted, confirms the conclusion that the vast majority of plants of the strains here cultivated, and under the conditions existing in local greenhouses and gardens, cannot be made to produce seed. BURBANK, on the other hand, reports that the *Richardia* as it grows wild in California matures seed in great abundance.<sup>3</sup>

The behavior of the artificially pollinated ovaries is peculiar. Ovaries not artificially pollinated shrivel in 10-15 days after reaching maturity. Pollinated ovaries behave otherwise. A large

<sup>3</sup> "The calla lily, in all its varieties, blooms and seeds in the utmost profusion here in California, setting big plump seeds in abundance. This includes all the yellow, white, and black varieties. I think the cause of not setting seeds in the east is wholly climatic."—From a private letter addressed to the writer.



FIGS. 34-39.—*Richardia africana*: fig. 34, immature carpel, containing developing ovule which is just beginning to show the anatropous character; fig. 35, primary archesporial cell; fig. 36, first nuclear division in formation of embryo sac; fig. 37, four-nucleate embryo sac; figs. 38, 39, mature embryo sac.

number of flowers were pollinated early in January. Three weeks later the ovaries had swollen to the size of large peas, and the gardener—a veteran in the growing of the *Richardia*—was confident that seed would be set. Within the following week most of these had begun to wilt, but a very few continued to grow until they were as large as small hazel nuts. Within two weeks almost all were withering, and from then on the molds rapidly made away with them. Five per cent or less of the pollinated plants produced seed.

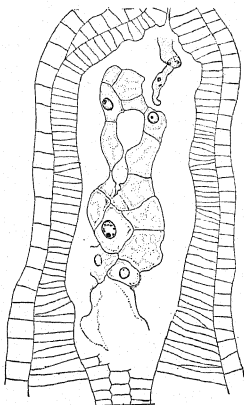


FIG. 40.—*Richardia africana*: disintegrating sterile nucellus and tip of pollen tube.

The explanation of this behavior is to be found in the fact that the greater number of ovules are congenitally sterile and develop no embryo sac. In some cases no cell can be discovered that can be definitely identified as a primary archesporial cell. In other cases it would seem that a potential archesporial cell makes its appearance, but fails to complete its normal course of development. The latter is to be inferred from the fact that the number of primary archesporial cells encountered in the very young material vastly exceeds the number of embryo sacs in the more mature material.

Sections of pollinated pistils containing sterile ovules demonstrated the fact that, in some cases at least, the pollen tube penetrates a sterile ovule, and reaches the tip of the nucellus. The fact that the pollinated sterile ovaries reach a considerably higher stage of development before withering than do unpollinated ovaries of the same sort would appear to be due to the stimulus of pollination. At all events, the writer can find no other cause to which it can reasonably be attributed. The direction of the pollen tube

into the ovule is probably determined by the presence of nutritive material in that quarter, since the nucellar cells, and even the tip cells of the integuments often become somewhat swollen and glandular and break down readily. If this be the case, the tube should enter a sterile ovule quite as readily as a fertile one. In a number of cases remains of shrunken pollen tubes were observed near or in contact with sterile nucelli (fig. 40).

The sterile nucellus finally breaks down, the process usually beginning within, and sometimes resulting in, the formation of an interior cavity before the disintegration of the outer cells (figs. 40, 41), and the nuclei of the cells occasionally lie free in this cavity before disintegration. The breaking down of the nucellus finally leaves the cavity within the integuments completely empty; the ovule soon collapses and begins to decay.

In the case of fertile ovules, the embryo sac is of the usual 8-celled type. The cavity soon becomes filled with a mass of thick-walled endosperm cells, and the endosperm is persistent in the seed. The proembryo develops a

little later than the endosperm, and is spherical in shape, finally lengthening out and developing a distinct notch (fig. 42).

Anthers dissected out, before the appearance of the flower bud as a swelling under the leaf-sheath, disclosed many large multinucleate mother cells. Tetrads are formed by successive divisions, the 4 daughter cells lying in the same plane. Material taken from a plant in which the flower bud was beginning to form a prominent swelling within the stem disclosed mature pollen grains filling the loculus of the anther. The latter has the usual thickened epidermal layer of rather small cells, inside which is an endothecium of large cylindrical cells with riblike thickenings. The tapetum consists

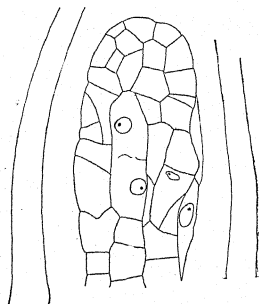
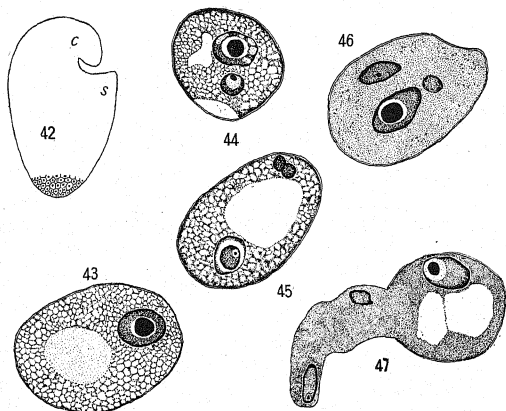


FIG. 41.—*Richardia africana*: sterile nucellus; disintegration just beginning.

of a layer 3 cells deep and disintegrates early. The spores lie in a mass of gelatinous material, the remains of the former tapetum, and scattered among them are many small, ill developed cells which show a tendency to break down. These are abortive pollen grains, or possibly in some cases abortive mother cells.



FIGS. 42-47.—*Richardia africana*: fig. 42, embryo in early stage of development; c, cotyledon; s, stem tip; fig. 43, mature pollen grain previous to nuclear divisions; fig. 44, pollen grain previous to division of generative cell; fig. 45, male cells in contact; fig. 46, tube nucleus and male cells, the latter abnormal as to shape and comparative size; fig. 47, sprouting pollen grain.

There is a great lack of uniformity in the divisions within the pollen grain. In one case (fig. 43) all were delayed until after dehiscence; but this is certainly very unusual. The first nuclear division, by which the generative nucleus is differentiated, usually occurs early. The generative nucleus does not surround itself with cytoplasm and a distinct cell membrane, but retains its nuclear character after division. Usually it divides before dehiscence occurs (fig. 45), although a number of grains were taken from

dehiscing anthers in which it was found that this division had not yet taken place. Grains were sprouted in weak solutions of glucose and lactose, and examined unstained, or stained in a weak aqueous solution of hematoxylin. One of these is indicated in fig. 47.

### Summary

1. The development of the archesporium in aroids follows no uniform rule. In one species studied,<sup>4</sup> the primary archesporial cell produces two megaspores, the outer one of which functions; in two cases there are four megaspores, the outermost one of which functions; in one case the primary cell produces two cells, one of which may be a tapetal cell; one species develops four megaspores in the same horizontal plane, and the functioning one then develops a tapetal cell at its upper end; one species develops four megaspores, the innermost of which functions; and in one case the primary sporogenous cell develops directly into an embryo sac.

2. The number of antipodals varies from 3 to 11, four species having regularly more than 3. This is a relatively primitive feature, and occurs in other primitive orders.

3. Previous to the work here reported, embryos of the spherical type lacking a suspensor had been reported by CAMPBELL in *Pistia*, *Dieffenbachia*, and *Lysichiton*. The writer has confirmed CAMPBELL'S observation with regard to *Dieffenbachia*, and has discovered the same sort of embryo in *Nephtytis*, *Philodendron*, *Arum*, *Aglaonema*, *Anthurium*, *Arisaema*, *Symplocarpus*, and *Richardia*. It seems clear that this type of embryo is characteristic of this family.

4. *Richardia* as here cultivated in the greenhouse has usually a sterile nucellus and does not develop an embryo sac. The disintegrating nucellar cells act as conducting tissue, and the pollen tube enters sterile nucelli, although gametic attraction cannot exist. The direction of the tube is conditioned on the presence of nutritive material.

5. Reduction of parts is indicated by the presence of staminodia in several of the species studied.

6. In an abnormal specimen supposed to belong to the genus

<sup>4</sup> GOW, JAMES ELLIS, Studies in Araceae. BOT. GAZ. 46:35-42, pls. 4-6. 1908.

*Stenospermation* functioning stamens replace the ovules in many of the carpellary chambers. This reminds one of a somewhat similar abnormality noted by CHAMBERLAIN in *Salix*, and of characteristics noted by TREUB in *Balanophora* and *Loranthus*.

7. In three species investigated with regard to number of chromosomes the homotypic number was found to be 16.

8. Tetrad formation in the anthers of some species is by means of simultaneous, in others by successive, divisions.

9. In *Richardia* the male nuclei are not surrounded by a definite cytoplasmic envelope, and the division producing them is often delayed until dehiscence or later. Sometimes the earlier divisions are similarly delayed.

10. The bulb of *Arisaema* may bear staminate and pistillate blossoms in alternate years.

Thanks are due to Mr. FRED J. SEAVER, of the New York Botanical Gardens, for furnishing most of the material on which the foregoing observations were made. The author also wishes to express his deep obligation to Professor T. H. MACBRIDE, of the State University of Iowa, for the suggestion which first led to the undertaking, and for advice and assistance freely rendered at every stage of the work.

CEDAR RAPIDS, IOWA



## SUMMER EVAPORATION INTENSITY AS A DETERMINING FACTOR IN THE DISTRIBUTION OF VEGETATION IN CONNECTICUT

GEORGE ELWOOD NICHOLS

For an area of its size, the state of Connecticut exhibits considerable diversity both in topography and in vegetation. From a geographical standpoint three well defined regions may be recognized: the Western Highland, the Eastern Highland, and the Central Lowland. The surface of the Highlands is for the most part exceedingly rugged, and in the northern part of the state, especially in the Western Highland, elevations of 300 or more meters are common. The Central Lowland is characterized, on the whole, by its gentler contours and lesser elevations. Over large areas here the surface is almost level, while the hills, as a rule, seldom reach a height of more than 75 meters. Exception to this latter statement, however, must be made in the case of the trap ridges which traverse the Lowland from north to south, dividing it lengthwise into two sections. In topography these conform with the Highlands rather than the Lowland. Geologically, as well as topographically, the Highlands contrast sharply with the Lowland. Except for limited areas of limestone, the Highlands are underlain by granites, gneisses, and schists. The Lowland rocks are sandstones and shales. Throughout the state, however, the underlying rock is in large part covered over by a mantle of glacial drift which in places is more than 50 meters in thickness.

The vegetational differences between various parts of Connecticut are not so sharply or clearly defined. Moreover, the situation is complicated by the fact that practically the entire area at one time or another has been deforested, while a large share of it is at present under cultivation, so that the nature of the original plant covering has become more or less modified. Considering the vegetation of the state in its entirety, the ultimate climatic formation is a forest dominated by various deciduous trees and hemlock. Of the virgin forest scattered remnants are occasionally encountered, but for the most part the present woodlands are second growth.

These undoubtedly differ in many respects from the original forests. Nevertheless, it seems probable, due to the sprouting capacity of the majority of the constituent trees, that in favorable cases, except for a decrease in the proportion of hemlock and a corresponding increase in the proportion of chestnut, the general aspect of the forest has not greatly altered during the last three centuries. The composition of this forest, however, was never uniform throughout the state. In northwestern Connecticut the original forest was of the so-called Northern hardwood type. Here the dominant trees were sugar maple, beech, and hemlock, associated with which were yellow birch, chestnut, and other hardwoods. Just how large a portion of the state was originally clothed by this type of forest is uncertain, but it undoubtedly was present throughout the greater part of the northern half of the Western Highland and extended southward toward the shore of Long Island Sound. In all probability it was also characteristic of at least the northern portion of the trap range and of the northernmost section of the Eastern Highland. Along the coast and throughout the greater extent of the Central Lowland, so far as can be determined, the most widely distributed type of original forest was composed largely of chestnut and various oaks. The trees characteristic of the northern hardwood forest were also present here, but were relatively less abundant and more restricted in their occurrence. In the forests of this area the tulip tree was frequently an important component. The original forests of the Eastern Highland, except in the northernmost part, would appear to have resembled more closely those of the coast and Lowland than those of the Western Highland. In the present forests the prominent trees are the oaks, especially the white and the red oak. Chestnut, while present, is much less conspicuous than in the Lowland forests. Hemlock, beech, and maple are of subsidiary importance.

There are other important and even more striking vegetational dissimilarities between various parts of the state. The discussion of these, however, is reserved for papers in course of preparation, in which the plants of the state are to be considered from an ecological standpoint.<sup>2</sup> It was the primary object of the experiments

<sup>2</sup> The first paper of this series, "The vegetation of Connecticut I. Phytogeographical aspects," has already been published (*Torrey* 13: 89-112. 1913).

to be described in the present paper to determine whether the distribution of the climax types of vegetation could in any way be coordinated with differences in the evaporating power of the air during the growing season. As is generally recognized, one of the greatest dangers that beset the growing plant is loss of water through transpiration. In ordinary plants, as soon as the amount lost in this manner exceeds that absorbed by the roots, the plant wilts. The rate at which transpiration goes on in any particular plant is regulated largely by the evaporating power of the surrounding air, and this in turn is dependent upon a complex of factors, such as humidity, temperature, direction and velocity of wind, etc. When exposed to uniform atmospheric conditions, however, it is known that the rate at which water is transpired by different kinds of plants varies, mesophytes transpiring more rapidly, xerophytes more slowly, and so on. That the great centers of plant distribution in various parts of the United States are directly related to well marked differences in the summer evaporation intensity has been ably demonstrated by LIVINGSTON,<sup>2</sup> while the earlier work of TRANSEAU<sup>3</sup> indicated an analogous correspondence between the precipitation-evaporation ratios for the entire year and forest distribution. It seemed possible, therefore, that in the area under consideration similar relations on a smaller scale might be detected.

Accordingly, with this object in view, during the summer of 1912 continuous evaporation records were taken at numerous localities in the state by means of porous clay cup atmometers of the type devised by LIVINGSTON.<sup>4</sup> For assistance in carrying out these experiments the writer is indebted to the various cooperators named below. The expense necessarily involved was in large part defrayed by Yale University. Altogether, 16 more or less widely separated stations were selected, and these were fairly uniformly distributed, through the three geographical regions of the state, 7 being located in the Western Highland, 3 in the Central Lowland, and 6 in the Eastern Highland. Three of the stations, one in each geographical region, were situated near the coast. The writer personally selected the sites and installed the atmometers at all the stations except

<sup>2</sup> Plant World 14:205-222. 1911.

<sup>3</sup> Amer. Nat. 39:875-889. 1905.

<sup>4</sup> Carnegie Inst. Publ. no. 50. 1906.

Storrs and Haddam. So far as possible, the sites were made to conform with one another, the instruments being placed in the open, where they would be freely exposed to the action of both sun and wind. Brief comment regarding the nature of the respective sites, together with the names of the various observers, is given in the following paragraph.

WESTERN HIGHLAND.—Salisbury: open hillside, north exposure, altitude 210 m.; Mrs. CHARLES S. PHELPS. North Colebrook: open field, slightly shaded in early morning and late afternoon, altitude 224 m.; station of particular interest on account of proximity to large tract of virgin northern hardwood forest; Mr. CARRINGTON PHELPS. West Cornwall (Cream Hill): hillside, west exposure, slightly protected from wind by trees, altitude 390 m.; Mr. C. L. GOLD. Litchfield: lawn in proximity of buildings, altitude 330 m.; Rev. JOHN HUTCHINS. Hawleyville: hillside lot, west exposure, slightly shaded in early morning and late afternoon, altitude 156 m.; Mr. C. B. HAWLEY. Collinsville: hillside lawn, west exposure, buildings in vicinity, altitude 135 m.; Mr. G. J. CASE. CENTRAL LOWLAND.—Hayden: open field, altitude 15 m.; site typical for neighboring tobacco plantations; Misses HELEN and GRACE CLAPP. Southington: lawn, in proximity of building; altitude 45 m.; Miss EUNICE MACKENZIE. EASTERN HIGHLAND.—Haddam: hill crest, altitude 144 m.; Professor A. L. DEAN. Storrs: open hillside, west exposure, altitude 195 m.; Professor L. A. CLINTON. Colchester: hillside cemetery, slightly shaded in late afternoon, east exposure, altitude 150 m.; Mr. H. P. BUELL. Danielson: hillside, west exposure, altitude 75 m.; Mr. F. E. BITGOOD. Voluntown: hill crest, altitude 82 m.; Mr. J. L. HERBERT. COASTAL REGION.—Niantic: lawn, in proximity of trees and buildings, slightly shaded in early morning, within 90 m. of sea shore, altitude 5 m.; Mrs. F. H. DART. New Haven: open lawn, slightly protected from wind, about 3 km. from shore, altitude 18 m.; G. E. N. Westport: hillside, about 0.5 km. from shore, altitude 30 m.; Mr. GRENVILLE MACKENZIE.

In each of the above mentioned localities a pair of atmometers was installed side by side, about 50 cm. apart. The bottles to which the cups are connected were set upon a T-shaped wooden support, being held in position by zinc casings made for the purpose. The base of the cup itself was thus raised about 56 cm. from the ground, or high enough to insure free circulation of the air about it. The entire apparatus was inclosed within a coarse-meshed chicken-wire cage. Of the two cups installed at the beginning of the season at each station, one was replaced by a fresh one after an interval of 5 weeks; the second original cup was renewed at the end of 10

weeks; and 5 weeks later a fourth cup was substituted for the one which at that time had been longest in operation. Distilled water was used in all cases. The majority of the instruments were set up during the last week of May and continued in operation until September 14. At the end of the season the cups were returned to the writer and restandardized,<sup>5</sup> the readings being then corrected in the usual manner,<sup>6</sup> and all results coordinated with those derived from standard cups. Except where for one reason or another particular cups were manifestly unreliable, the records of the two were averaged to obtain the accepted readings.

In this way a practically complete set of weekly readings throughout the season has been tabulated for nearly every station. Such a series of figures brings out a number of interesting facts. It is found that for the state as a whole the maximum weekly rate of evaporation for the season occurred during the first week in July, when there was an average water loss from each cup of 211 cc. During this week the instruments at Hayden evaporated 256 cc., and at 5 other inland stations in the Lowland and Eastern Highland the rate exceeded 225 cc., while at Niantic there was a loss of but 144 cc. At no time during the season did the amount evaporated during the recorded week at any of the stations in the Western Highland or in the Coastal Region amount to as much as 225 cc. The lowest rate of evaporation throughout the state was observed during the first week in September, when the average was 41 cc., the minimum being reached at Niantic (22 cc.). The mean weekly evaporation rates for the state as a whole, based on the averages of all the stations, were as follows:

June 1-June 8	146 cc.	July 27-Aug. 3	121 cc.
“ 8- “ 15	178 “	Aug. 3-Aug. 10	113 “
“ 15- “ 22	124 “	“ 10- “ 17	108 “
“ 22- “ 29	153 “	“ 17- “ 24	53 “
“ 29-July 6	211 “	“ 24- “ 31	125 “
July 6- “ 13	155 “	“ 31-Sept. 7	41 “
“ 13- “ 20	119 “	Sept. 7- “ 14	104 “
“ 20- “ 27	170 “		

<sup>5</sup> See NICHOLS, G. E., A simple revolving table for standardizing porous cup atomometers. *BOT. GAZ.* 55:249-251. 1913.

<sup>6</sup> See *Plant World* 13:111-115. 1910.

There seems to be no obvious advantage, so far as general considerations are concerned, in setting forth in detail the results for each week in each locality. It has rather seemed simpler to give local averages for longer periods, a method already adopted by LIVINGSTON<sup>7</sup> in presenting similar data. The periods selected correspond approximately to the three summer months—June, July, August—and the first half of September. The mean weekly evaporation rates for the various stations during these periods are given in table I.

TABLE I

MEAN WEEKLY EVAPORATION RATES IN CC., AS RECORDED BY THE POROUS CUP ATMOMETER, FOR 16 STATIONS IN CONNECTICUT, FROM JUNE 1 TO SEPTEMBER 14, 1912. FIGURES IN PARENTHESES INDICATE NUMBER OF WEEKS FOR WHICH READINGS ARE RECORDED, IF LESS THAN THE STATED PERIOD

Station	June 1 to June 29	June 29 to Aug. 3	Aug. 3 to Aug. 31	Aug. 31 to Sept. 14	Average for season	June 1 to Aug. 3
Salisbury.....	152	168	109	69	134	161
Colebrook.....	133 (2)	119	87	53	100 (14)	122 (7)
Cornwall (Cream Hill).....	115	130	79	65	103	123
Litchfield.....	113	147 (4)				130 (8)
Hawleyville.....	119	141	113	89	121	131
Collinsville.....	144	161	98	71	128	154
Hayden.....	165	182	124	77	148	174
Southington.....	170	167	109	70	140	168
Haddam.....	197	184	99	67	149	189
Storrs.....	182	175	100	97	146	178
Colchester.....	157	156	99	79	131	157
Daniels.....	181	173	100	101	143	172
Voluntown.....	176	165	112	81	143	170
Niantic.....	104	105	71	40	87	104
New Haven.....	146	145	102	68	123	146
Westport.....	142	165	107	71	134	156

In considering the evaporation intensity of the air as a possible factor affecting the distribution of the vegetation in this region, it seems reasonable to assume that its effect on plants is felt most keenly during the earlier part of the growing season, at a time when growth and development are taking place most rapidly and when the immature tissues and organs are as yet inadequately protected from excessive transpiration. An examination of the first four

<sup>7</sup> Plant World 14:205-222. 1911.

columns of figures in table I reveals the fact that during the first two monthly periods the mean weekly rate of evaporation far exceeded that maintained during the latter part of the season. In other words, the period of maximum evaporation coincided approximately with the more critical period of vegetative activity. In view of this correlation, coupled with the natural assumption that it is the periods of excessive evaporation that are most influential in determining the character of vegetation, it has been thought best in drawing conclusions to disregard entirely the data obtained during the latter part of the season, when for the most part a uniformly low rate of evaporation prevailed throughout the state, and to base deductions on the observations of these first two months. The mean weekly rates of evaporation obtaining at the various stations from June 1 to August 3 have therefore been indicated in the last column of table I. With these figures as a basis, it is a simple matter to compute approximately the relative evaporating power of the air for the various geographical and vegetational regions of the state during the period of combined maximum vegetative activity and evaporation intensity for the year 1912. Upon averaging the results for the inland stations of the Highlands and Lowland and of the stations along the coast, it is found that the weekly water loss, as recorded by the porous cup atmometer, was as follows: Western Highland, 137 cc.; Central Lowland, 171 cc.; Eastern Highland, 173 cc.; Coastal Region, 135 cc. It would thus appear that the area dominated largely by the mesophytic northern hardwood type of forest, and the strip along the coast, constitute areas of relatively low evaporation intensity; and that the rate of evaporation in the Eastern Highland, where oaks predominate in the forest, is somewhat higher than that in the Central Lowland, where the more mesophytic chestnut is the character tree. The relatively low evaporation rate along the coast was not wholly unexpected and will be referred to again. Explanation for the gradual diminution in evaporation intensity apparent in passing from west to east along the coast may be looked for in the fact that while the western part of the shore is shut off from the ocean by Long Island, the eastern portion is more exposed and therefore possesses a more maritime climate.

In the foregoing paragraphs no account whatever has been taken of the varying amounts of precipitation occurring in different sections of the state. But since the amount of water present in the ground and therefore available for plant use is in itself a potent factor in determining the character of vegetation, and since its abundance is so largely controlled by the amount of precipitation, it is necessary, in order to gain a comprehensive view of the situation, that the relationship between the phenomena of precipitation and evaporation within the area under discussion be considered. So far as observed, there is no constant ratio between the rate of precipitation and the intensity of evaporation. Thus the rate of evaporation during a heavy rain is hardly lower than during a dense fog. But the ratio between the *amount* of precipitation and that of evaporation, especially over considerable periods of time, is of vital significance. In the state of Connecticut there is an average annual precipitation of 120.9 cm. (47.59 inches).<sup>8</sup> This is distributed approximately as follows: Western Highland (inland), 126.8 cm. (49.94 in.); Central Lowland (inland), 119.6 cm. (47.08 in.); Eastern Highland (inland), 117.7 cm. (46.35 in.); Coastal Region, 116.8 cm. (45.98 in.). Of more special interest, however, for the purpose of comparison with the observed rates of evaporation, are the amounts of rainfall that occurred in the various localities during the past season for the period extending from June 1 to August 3. These, together with the contemporaneous evaporation records, are therefore indicated in table II. For convenience in comparing the two sets of data, the evaporation readings, heretofore given in terms of cc., are here expressed in units of depth as well, the conversion being made with reference to a more or less arbitrarily chosen free water surface.<sup>9</sup>

An examination of table II shows very convincingly that, for the period under observation, nowhere in the state would the amount of rainfall have compensated for the amount of water which would have been evaporated from a free water surface during

<sup>8</sup> Based on records from 14 scattered stations. This and the following figures regarding precipitation have been computed from statistics published in the *Monthly Weather Review* of various dates.

<sup>9</sup> The standard water surface on which these reductions are based is that used by LIVINGSTON (see *Plant World* 14:214, 215, 1911).



the same interval. As is indicated in the last column, the disparity was least in the Western Highland, greatest in the Eastern Highland. The fact that, despite this at first sight rather startling deficiency in precipitation as compared with evaporation during this particular season, plants were still able to maintain the balance between absorption and transpiration may be attributed partly to the manner in which evaporation is modified by the physical structure of various soils, partly to the adequacy of the ground-water supply, which in turn is largely dependent on the influx of water

TABLE II

MEAN WEEKLY RATES OF RAINFALL AND EVAPORATION DURING THE PERIOD OF  
9 WEEKS FROM JUNE 1 TO AUGUST 3, 1912

	MEAN WEEKLY RAINFALL		MEAN WEEKLY EVAPORATION			RATIO BETWEEN RAINFALL AND EVAP- ORATION
	cm.	in.	cc.	cm.	in.	
Western Highland...	1.19	0.47	137	1.94	0.76	0.61
Central Lowland...	1.14	0.45	171	2.42	0.95	0.47
Eastern Highland...	1.02	0.40	173	2.45	0.96	0.42
Coastal Region....	0.91	0.36	135	1.91	0.75	0.48

at times of the year when the effects of evaporation are less pronounced, and partly to various structural peculiarities of the plants themselves by which transpiration is regulated. Nevertheless, the marked excess in the rate of evaporation over that of precipitation at such a critical period in the life of the plant cannot but make some impress on the character of the vegetation. Another interesting fact is brought out in this table. As has already been noted, the absolute amount of evaporation in the Coastal Region is even lower than that in the Western Highland. But taking the ratio between the amount of evaporation and that of rainfall as a criterion, it is seen that conditions in the Coastal Region approximate more closely those in the Central Lowland. Similarly the less mesophytic nature of the Eastern Highland is forcibly accentuated.

It is fully appreciated that too definite conclusions cannot be drawn from such a series of experiments conducted for but a single season. It is recognized, furthermore, that it is well-nigh impossible to select with certainty in different localities representative sites

in which the instruments will be exposed to absolutely parallel atmospheric conditions. The danger in placing too much reliance on a single set of data for a given locality was impressed upon the writer by results secured from a supplementary series of instruments which was operated in various plant habitats in the vicinity of New Haven for the last five weeks of the season. During this period the mean weekly evaporation rate at the central station averaged 86 cc. Instruments in the other sites averaged as follows: exposed summit of trap ridge, about 6 km. from coast, altitude 81 m., 138 cc.; salt marsh, 128 cc.; lee of low sand dunes along shore, about 100 m. removed from salt marsh station, about 131 cc.; open bog, altitude 6 m., 81 cc. More accurate conclusions as to the evaporation intensity prevailing throughout the state might of course be derived from a series of instruments placed in diverse habitats in each of the localities selected and operated for several seasons, but at the present time no further investigation along these lines is contemplated. And while the data obtained from the experiments of this one season do not permit final conclusions, they at least suggest that the evaporation intensity of the air may be a factor of no little import in determining the character of the vegetation in different parts of Connecticut.

YALE UNIVERSITY

# CURRENT LITERATURE

## BOOK REVIEWS

### Soil acidity

The injury to vegetation by smoke from factories and smelters is well known. Much has been written on the subject, and considerable experimental work is now under way in various parts of the world. WIELER<sup>1</sup> has published recently an account of one series of experiments, begun in 1905, dealing with plant growth and the lack of lime in the soil. In this account he defends the thesis that smoke and smelter fumes are injurious to plants, not because of their direct effect on the leaves, but because the acid-forming substances they contain are absorbed in the soil and there neutralize the lime so necessary to plant growth. He argues also that there is an injurious effect on the micro-organisms of the soil.

The region studied in greatest detail is a part of the Innerste Valley where considerable damage has been done by fumes from the Frankenscharn smelters. The forest here has been driven back to considerable distances, amounting to 3.5 kilometers in some directions. Even where trees were still standing, they showed by their stunted appearance and yellow leaves or needles the injurious effects of conditions surrounding them. Certain slopes bore only heath, low shrubs, and stunted trees, others only grasses, and still others no vegetation at all. Injury was greatest close to the smelter and decreased as the distance from the smelter increased.

On the three kinds of slopes just described, experimental plots were laid out at distances of 0.5 to 1.5 kilometers from the source of the fumes. To these plots additions were made of lime, lime and ammonium hyperphosphate, and ammonium hyperphosphate alone. This substance was added on the supposition that the soil was poor in nitrogen. No striking results were obtained by its use, however, and it need not be considered further. Slaked lime was applied in finely ground form in quantities varying from 50 to 100 kilograms per hectare. Untreated plots were studied as checks in all cases.

Plantings were made of *Picea excelsa*, *Pinus sylvestris*, *P. montana*, *Quercus robur*, *Fagus sylvatica*, *Betula alba*, *Vicia villosa*, and *V. sativa*. It was found in all cases that plants on unlimed plots grew much less rapidly and appeared less healthy than those on limed plots. Conifers and legumes either did not grow at all without lime, or only poorly. Oak, beech, and birch proved to be less rigorous in their requirements, but did better on treated plots. Plants from seed gave poorer results than those transplanted from the nursery.

<sup>1</sup> WIELER, A., Pflanzenwachstum und Kalkmangel im Boden. 8vo. pp. vii+235. figs. 43. 1912.

Mention was made above of the yellow color of leaves on plants in affected areas. This condition is generally considered to be due to a lack of nitrogen in the soil; this in turn is a consequence of poor development of the bacterial flora. That such conditions prevail in the soil of these denuded or partly denuded hillsides was shown by the fact that in unlimed plots very few bacterial nodules were found on the roots of legumes. Development of such nodules was practically normal, however, where lime had been added.

Root systems were found to be small on unlimed soil. Both primary and secondary roots were short and there was only a small amount of branching. As a consequence, the whole root system formed a knob or lump which came in contact with very little soil and furnished poor anchorage for the plant. There was thus more danger of injury from drought and from strong winds. Results confirming those already described were obtained by liming soils in other localities, hence they need not be discussed here.

The author's idea of the causes of the deliming of soils and consequent inhibition of plant growth has already been stated. In a discussion of his results he considers these causes at some length. He finds, in the first place, that all the untreated soils really are poor in lime. Near the smelter this substance was present to the extent of 0.012 per cent; in experimental plots along the sides of the valley it varied from 0.017 per cent to 0.045 per cent; under a spruce stand it was 0.038 per cent.

Further investigation of these soils brought out the surprising fact that they were acid in reaction, instead of alkaline or neutral, as might have been supposed. It is known of course that wet moor soils are acid, but this has been found by BAUMAN and SULLY to be due, not to the presence of free humic acids, but to the power of certain substances in these soils to decompose salts by forcing acids out of combination and by absorbing the bases with which they were combined. These authors showed further that this reaction is brought about by the "absorptively unsaturated" condition of the cell walls of sphagnum. The acid character of moor soils depends, therefore, on the fact that the material from which they arise is already acid. But there is good reason, says WIELER, for thinking that dry peaty soils originate in the same way as do those of sphagnum bogs; that is, the plant remains in the former case are acid, just as they were in the latter. This deduction was amply confirmed by tests on living leaves and needles, the same organs dead and still remaining on the tree, or covering the ground beneath these trees.

That the acids set free in the manner indicated above are not neutralized or decomposed depends finally on the failure of microorganisms to do their usual work. From undecomposed organic remains acids are being washed out continually by rains. As soon as bacterial and fungal action begins these acids are broken up into harmless substances. In soils, however, where the acid content is steadily increasing, as in the region around the Frankenscharn smelter, the bases, most important of which is lime, are neutralized. As a consequence, microorganisms do not thrive, and plant fragments and the soil

itself remain acid. And not only so, but the important process of nitrification, carried on by bacteria whose activity is closely connected with the amount of lime available, is seriously retarded. Leaves become yellow and there is a general nitrogen starvation of all vegetation.

It developed during the course of the investigation that the addition of lime to such soils, even though it improved materially the conditions for plant growth, reduced the acidity only one-third. This result, taken together with the fact that an acid soil need not contain free acid, and the further fact that forest trees do grow on acid soils, makes it plain, in the opinion of the author, that lime is valuable, not because it neutralizes acids, but because it furnishes a substance indispensable to normal plant growth. The conclusion thus reached points definitely to the use of lime as a means of combating smoke injury to vegetation and of rendering denuded areas again able to support plant life.

A supplementary investigation of the effect of metallic poisons in the soil showed that the sensitiveness of plants to these poisons varies greatly. It was found possible to arrange a complete series, leading from those which were seriously affected to those whose growth was definitely improved.—D. H. ROSE.

### The living plant

It is with some interest that plant physiologists and ecologists will read GANONG'S<sup>1</sup> new book on *The living plant*. It is the first attempt in English to bring, in a comprehensive way, the main findings of these subjects within reach of the layman. The aims of the volume are well stated in the first paragraph of the preface: "It is not designed as a digest of our present scientific knowledge of plant physiology for the use of experts in the subject, but, in conformity with the aim of the series of which it is a part, it seeks to present to all who have interest to learn an accurate and vivid conception of the principal things in plant life. I was once myself such a learner, and I have tried to write such a book as I would then have delighted to read. It is, in a word, an attempt at that literature of interpretation which was forshadowed by FRANCIS BACON in the fine passage that stands on its dedicatory page."

Aside from the general interest in plants, we have at present a rapidly growing interest in agriculture. This makes the issue of the present clear statement of the principles of plant production especially timely. In this work the author has lived up to his high reputation as a teacher. One is surprised at the clearness and vividness with which he sets forth the main features of plant activity. Aside from presenting the main findings of the subject, the author gives a clear insight into the scientific method in action, for repeatedly he shows the processes by which the conclusions have been reached. He likewise makes clear the large cosmic relations of the subject.

<sup>1</sup> GANONG, WILLIAM F., *The living plant*. 8vo. pp. xii+148. figs. 178. New York: Henry Holt & Co. 1913.

Citation of a few of the 18 chapter headings will give an idea of the scope and perhaps the viewpoint of the work: (i) "The various ways in which plants appeal to the interests and mind of man"; (ii) "The prevalence of green color in plants, and the reason why it exists"; (iii) "The profound effect on the structure of plants produced by the need of exposure to light"; (iv) "The kinds of work that are done by plants, and the source of their power to do it"; (xii) "The many remarkable arrangements by which plants secure union of the sexes"; (xvii) "The remarkable improvement made in plants by man, and the way he brings it about."

The author pronounces himself a vitalist of a worthy type, "perfectly natural vitalism based on the superior interpretive power of an hypothesis assuming the existence in nature of an X-entity, additional to matter and energy, but of the same cosmic rank as they." This is contrasted with "a supernatural vitalism of the theological type." One wonders whether the X-entity as defined above would not satisfy any of our present theologians, and whether the distinction is not a matter of words rather than a real difference. It should be stated that the X-entity is called in here only to explain the mechanics of development and inheritance. As a matter of fact, the safe position here is that of the agnostic; for we certainly do not know, in spite of assumption and positive statement to the contrary. Nor should we be discouraged by the fact that we have not made great progress in the physico-chemical explanation of development and inheritance, for the fundamental physics and chemistry of the material here involved are little developed. The serious study of the chemistry of proteins has nearly all been within the last two decades, and that of the physics of colloids within a decade. Every treatise on these subjects points out much more that is unknown than is known. The strides we are making in these fields, along with a phase of work that is now only beginning, namely, application of the methods of protein and colloidal physics and chemistry to the study of protoplasm, promise great progress in the immediate future. Here also we should not lose sight of the great contribution of KLEBS and other experimental morphologists. These are the days of hopeful agnosticism in physiology.

One is disappointed at the overworking of adaptation in the book. An adaptational explanation is apparently placed coordinate with the physical or chemical explanation. This may make the book attractive to laymen, but it hardly expresses the present spirit of plant physiology. The book also contains many statements not abreast of our present knowledge. The following sentence savors of INGENHOUS' original statement: "It vitiates the air by its respiration, but in the long run purifies it still more by its photosynthesis." I believe it is now fully proved that vitiated air does not result from increased carbon dioxide content. Alcoholic fermentation of the yeast is spoken of as giving a copious release of energy available for growth. Also the yeast is said to be unable to respire in any other way. Per weight of sugar used, alcoholic fermentation releases about one-twentieth the energy released by aerobic

respiration, and growth does not generally occur in the yeasts in absence of aerobic respiration. One is also surprised at the author's slighting remark concerning the study of other products of alcoholic fermentation aside from carbon dioxide and alcohol, especially when he calls to mind EHRlich's recent important contribution on this point.—WILLIAM CROCKER.

#### NOTES FOR STUDENTS

**Current taxonomic literature.**—O. AMES (Philipp. Jour. Sci. Bot. 7:125-143. 1912), in continuation of his studies on Philippine orchids, lists 54 species of the genus *Bulbophyllum*, 19 of which are new to science.—A. BERGER (Monats. für Kakteenk. 22:147, 148. 1912) has published a new species of *Opuntia* (*O. tomentella*) endemic in Guatemala.—A. D. BETTS (Ann. Bot. 26:795-799. pls. 75, 76. 1912) describes and illustrates a new genus and species of bee-hive fungus (*Pericystis alvei*). The fungus grows on the pollen stored in the honey-comb.—E. P. BICKNELL (Bull. Torr. Bot. Club 39:415-428. 1912) in a tenth article on "The ferns and flowering plants of Nantucket" records further data concerning the Nantucket flora and describes two new species (*Linum intercursum* and *Ilex fastigiata*).—G. BITTER (Rep. Nov. Sp. 11:1-18, 202-237, 349-394. 1912) in continuation of his studies in the Solanaceae has published several new species and varieties from Central and South America.—F. BÖDEKER (Monats. für Kakteenk. 22:152-155. 1912) describes and illustrates a new species of *Mamillaria* (*M. Verhaertiana*) probably indigenous in Mexico.—J. BROADHURST (Bull. Torr. Bot. Club 39:357-385. pls. 26-29. 1912) in continuation of her studies in the genus *Struthiopteris* records 15 additional species, 3 of which are new to science, the others being transfers from *Lomaria* or *Blechnum*.—N. E. BROWN (Kew Bull. 28L. 1912) describes a new genus (*Thorncroftia*) of the Labiatae from South Africa.—E. CHIOVENDA (Ann. di Botanica 10:383-415. 1912) under the title "Plantae novae vel minus notae e regione aethiopica" has published several species of flowering plants new to science and proposes the following new genera: *Spathulopetalum* of the Asclepiadaceae and *Negria* of the Gramineae.—T. D. A. COCKERELL (Torreya 12:244-247. 1912) in an article entitled "*Tragopogon* in Colorado" finds four recognizably distinct forms of this genus in Colorado, including a new hybrid (*T. porrifolius* × *dubius*).—W. G. CRAIB (Kew Bull. 266. 1912) describes a new genus (*Murtonia*) of the Leguminosae from Siam.—E. L. EKMAN (Arkiv für Botanik 11, no. 4. pp. 61. pls. 1-4. 1912) under the title "Beiträge zur Gramineenflora von Misiones" includes 5 new species of grasses from Argentina.—F. FEDDE (Rep. Nov. Sp. 11:196, 197. 1912) describes 2 new species of *Corydalis* from western North America.—M. L. FERNALD (Rhodora 14:188-190. 1912) discusses the inland loose-flowered roseate form of "hardhack" and designates it as *Spiraea tomentosa* var. *rosea* (Raf.) Fern.; the same author (*ibid.* 192) also characterizes a hitherto unrecorded form of ash, namely *Fraxinus americana* f. *iodocarpa* Fern.—L. N. GOODING (Muhlenbergia 8:92-94. 1912) under the title "New southwestern ferns" describes 5 new species and one variety from Arizona, New Mexico, and

Sonora.—E. L. GREENE (Leaf. Bot. Obs. and Crit. 2:229-275. 1912) has described 63 new species of flowering plants, chiefly from western North America; these pages with title-page and index close the second volume. The same author (Rep. Nov. Sp. 11:108-111. 1912) under the heading "Novitates Boreali-Americanae VI" has published 7 new species in the genus *Cercis* from the southern and western states, and (Am. Mid. Nat. 2:290-296. 1912) under the title "Western meadow rues I" describes 7 new species of the genus *Thalictrum*, and also (*Muhlenbergia* 8:117-119. 1912) records 5 new species of *Lupinus* from Oregon and California.—J. M. GREENMAN (Field Mus. Nat. Hist. Bot. Ser. 2:323-350. 1912) has published about 40 new species and varieties of spermatophytes, mainly from Mexico and the West Indies. One new genus (*Shafera*) of the Compositae from Cuba is included.—E. HACKEL (Rep. Nov. Sp. 11:18-30. 1912) has published 14 new species and several varieties of grasses, mainly from Bolivia, based on the collections of Dr. O. BUCHTIEN.—E. HASSLER (*ibid.* 165-178) has published new species and varieties of Compositae and Aristolochiaceae from Paraguay.—A. A. HELLER (*Muhlenbergia* 8:85-91, 103-107, 109-116. pls. 11, 12. 1912) describes 7 new species of *Lupinus* and (*ibid.* 132) a new *Mimulus* (*M. micranthus*) from the Pacific coast region.—F. T. HUBBARD (*Rhodora* 14:165-173, 184-188. 1912) writing on "Nomenclatorial changes in Gramineae" calls attention to the names applied to certain species of grasses in the seventh edition of GRAY'S *Manual* as being at variance with the International rules of botanical nomenclature. The corrections number seventeen, and five of these are new combinations; the changes effected are mostly in the genus *Panicum*.—C. LAUTERBACH (Bot. Jahrb. 49:1-169. 1912) in cooperation with several specialists under the title "Beiträge zur Flora von Papuasien I" has published the first of a proposed series of articles dealing with the flora of New Guinea. The present article contains descriptions of upward of 125 species and varieties new to science, and the following new genera are proposed: *Andruris* of the Triuridaceae, *Papualthia*, *Oncodostigma*, *Oreomitra*, and *Schefferomitra* of the Anonaceae.—H. LÉVEILLÉ (Bull. Géogr. Bot. 22, IV, 217-224. 1912) gives a synoptical revision of the genus *Circaea*, recognizing 5 species, and several varieties and forms. The same author (Rep. Nov. Sp. 11:63-67. 1912) under the title "Decades plantarum novarum" has published several new species of flowering plants from Asia and includes a new genus (*Cavaleria*) of the Hamamelidaceae.—F. L. LEWTON (Smiths. Misc. Coll. 60, no. 4. pp. 1, 2. pls. 1, 2. 1912) describes a new species of *Gossypium* (*G. irenaeum*) from Guatemala. The same author (*ibid.*, no. 5, pp. 1-4. pls. 1-5) proposes a new genus (*Kokia*) of the Malvaceae, and (*ibid.*, no. 6. pp. 1-10. pls. 1-5) records a new species of *Gossypium* (*G. Hopi*), the cotton of the Hopi Indians.—G. LINDAU (Rep. Nov. Sp. 11:122-124. 1912) in an article entitled "Einige neue Acanthaceen" describes 3 new species in this family from Panama.—A. LINGELSHEIM (Mitteil. Thür. Bot. Ver. 29:48, 49. 1912) has published a new species of *Acalypha* (*A. striolata*) from Brazil.—T.



LOESENER (Verhdl. Bot. Ver. Prov. Brdbg. 53:50-86 [215-251]. 1912) in a seventh article on "Plantae Selerianae" continues the enumeration of plants collected in Mexico and Central America by C. and E. SELER. Several species and varieties new to science are included.—W. H. LONG (Mycologia 4:282-284. 1912) describes a new species of rust (*Peridermium inconspicuum*) found on *Pinus virginiana* at Glen Echo, Maryland, and records a new generic type (*Tricella*) collected on *Coursetia glandulosa* Gray in Sabina Canyon, Santa Catalina Mountains, Arizona.—J. LUNELL (Am. Mid. Nat. 2:287-290, 301, 302. 1912) describes 3 new species and 2 varieties of flowering plants from North Dakota.—E. D. MERRILL (Philip. Jour. Sci. Bot. 7:227-251. 1912) under "Nomenclatural and systematic notes on the flora of Manila" records important data concerning the flora of the Philippines and describes 6 species new to science.—J. A. NIEUWLAND (Am. Mid. Nat. 2:299, 300. 1912) proposes the establishment of the Rafinesquean names *Agaloma* and *Lepadena* and transfers thereto several species hitherto passed by most authors as members of the genus *Euphorbia*.—C. H. PECK (N.Y. State Mus. Bull. 157:5-139. pls. 124-130. 1912) in the "Report of the state botanist for 1911" under different subheadings records important data concerning particularly the fungus flora of New York and includes descriptions of about 50 new species and varieties of flowering plants.—J. PERKINS (Bot. Jahrb. 49:170-176. 1912) in cooperation with noted specialists has published the first part of a paper on "Beiträge zur Flora von Bolivia." Descriptions of 6 new species of mosses are included.—A. PULLE (Recueil. Trav. Bot. Néerl. 9:125-169. pls. 2, 3. 1912) under the title "Neue Beiträge zur Flora Surinams III" in cooperation with specialists has published several new species of flowering plants from South America. One new genus (*Clavapetalum*) of the Icacinaceae is included.—J. A. PURPUS (Monats. für Kakteenk. 22:148-150, 161-164. 1912) in continuation of his work on the Cactaceae has published 7 new species from Mexico.—A. PUTTEMANS (Bull. Soc. Roy. Bot. Belg. 48:235-247. 1912) in an article entitled "Nouvelles maladies de plantes cultivées" has described new species of fungi (*Oidium Begoniae*) found on leaves of *Begonia*, and *Cercospora Chrysanthemi* on leaves of *Chrysanthemum*; both host plants were in cultivation near Rio de Janeiro, Brazil.—C. R. W. K. VAN ALDERVERELT VAN ROSENBERG (Bull. Jard. Bot. Buit. Ser. II, 1-41. pls. 1-5. 1912) on "New or interesting Malayan ferns 4" describes several new species and proposes a new genus, namely *Scleroglossum*.—E. ROSENSTOCK (Rep. Nov. Sp. 11:53-60. 1912) has published 12 new species and 9 varieties of ferns, based on collections made in Bolivia by Dr. O. BUCHTIEN.—H. H. RUSBY (Bull. N.Y. Bot. Gard. 8:89-135. 1912) in a second paper on "New species from Bolivia collected by R. S. WILLIAMS" describes 113 species as new to science.—P. A. RYDBERG (Bull. Torr. Bot. Club 39:301-328. 1912) under "Studies on the Rocky Mountain flora XXVII" describes about 30 new species of flowering plants and makes several new combinations.—W. E. SAFFORD (*ibid.* 501-508) under the title

"Desmos the proper generic name for the so-called Unonas of the Old World" revives the name *Desmos* of LOUREIRO and refers thereto 16 species, all of Old World distribution.—J. H. SCHAFFNER (Ohio Nat. 13:19-21. 1912) records a new species of *Equisetum* (*E. kansanum*) from Kansas.—L. SCHKORBATOW (Ber. Deutsch. Bot. Gesells. 30:474-482. 1912) under the title "Zur Morphologie und Farbstoffbildung bei einem neuen Hyphomyceten" describes a new genus and species of fungus, namely *Gemmophora purpurascens* obtained from laboratory cultures.—R. SCHLECHTER (Orchis 6:112-119. pls. 25-26. 1912) has published several new species of orchids, including 4 from South America. The same author (Rep. Sp. Nov. 11:41-47. 1912) records further new species in this family and proposes a new genus (*Xerorchis*) from Brazil, and (*ibid.* 147-150) reestablishes the generic name *Aa* Rchb. f. and refers thereto 15 species of South American orchids which have passed hitherto under *Altensteinia*.—W. A. SETCHELL (Univ. of Calif. Publ. Bot. 4:229-268. pls. 25-31. 1912) under the title "Algae novae et minus cognitae I" discusses 14 species, proposing 4 new combinations, 8 new species, and 3 new genera (*Hapterophycus* in Ralfsiaceae, *Besa* in Gigartinaceae, and *Baylesia* in Dumontiaceae).—C. SKOTTSBERG (Bot. Jahrb. 48:Beibl. 107. pp. 17-26. 1912) has published a new species of *Tetrachondra* (*T. patagonica*) from Patagonia. Hitherto the genus has been considered monotypic, occurring only in New Zealand.—O. STAFF (Kew Bull. 278. 1912) has published a new genus (*Farquharia*) of the Apocynaceae from tropical Africa.—J. STUCHLIK (Rep. Nov. Sp. 11:151-162. 1912) under the title "Zur Synonymik der Gattung *Gomphrena* II" has published several new species and varieties of this genus from Mexico and South America.—R. THAXTER (Proc. Am. Acad. Arts and Sci. 48:155-223. 1912) in a paper entitled "New or critical Laboulbeniaceae from Argentina" describes nearly 70 species new to science and proposes the following new genera: *Mimeomyces*, *Tetrandromyces*, *Autophagomyces*, *Cryptandromyces*, *Synandromyces*, *Zeugandromyces*, *Scaphidiomyces*, *Scelophoromyces*, and *Synaptomyces*. The same author (*ibid.* 365-386) under "Preliminary descriptions of new species of *Rickia* and *Trenomyces*" has described 18 new species of the former genus and 4 of the latter.—I. TIDESTROM (Proc. Biol. Soc. Wash. 26:13. 1913) has published a new species of *Salicornia* (*S. utahensis*) from Tooe Valley, Utah.—P. VUILLEMIN (Bull. Soc. Bot. Fr. IV. 12:34-40. pl. 1. 1912) describes and illustrates a new genus (*Beauveria*) of the Verticillaceae, based on *Botrytis Bassiana* Balsamo.—C. WARNSTORF (Philip. Jour. Sci. Bot. 7:253-258. 1912) contributes an article on "Die Sphagna der Philippinen" and adds a new species of this genus from the Island of Luzon.—H. F. WERNHAM (Jour. Bot. 50:241-244. pls. 520, 521. 1912) under the title "New Rubiaceae from tropical America I" has published several species new to science and proposes two new genera, namely *Carmenocania* and *Pseudohamelia* from Colombia and Ecuador.—G. S. WEST (*ibid.* 321-331), in continuation of his studies in the algae, records important data and proposes a new genus (*Scourfieldia*) of the Volvocaceae from Essex, England.—J. M. GREENMAN.

**General biology of rusts.**—Of more than ordinary biological interest is a paper by TISCHLER<sup>3</sup> on the relation between *Uromyces Pisi* and its acedial host, *Euphorbia Cyparissias*. As is well known, the infection of *E. Cyparissias* by that rust takes place in the buds of the subterranean shoots in which the mycelium persists during the winter. The shoots which arise from such infected buds in the following spring show the characteristic deformations caused by the rust, but occasionally shoots are observed which outgrow the disease and develop normal leaves on their upper portion. This behavior led TISCHLER to investigate at what stage in their transition from embryonic to mature tissue the cells are subject to the formative influence of the fungus, and also to what extent such influence reaches beyond the area actually invaded by the mycelium. He found that the growing points of infected plants could be freed from the fungus by keeping the plants at a high temperature or under other conditions favoring rapid development. Under such conditions the newly developed parts of the shoots are normal. The emancipation of the growing apex from the fungus succeeds more readily as the fungus approaches the fruiting stage, and after the acedia are mature normal branches frequently develop from the infected plants if they retain sufficient vitality. Conversely, when the formation of acedia is suppressed (by keeping the plants in the dark), it is not possible to free the shoots from the fungus. The fungus appears to be incapable of further development after it has reached the fruiting stage.

These experiments show that the meristematic tissue of the growing point is not subject to the formative influence of the fungus, but that such influence must be exerted on cells which are no longer embryonic; nevertheless a histological examination shows that the mycelium is present even among the outer layers of embryonic cells. Here, however, the mycelium is entirely intercellular, no haustoria being formed. As soon as the cells lose their strictly embryonic character, that is, as soon as vacuoles appear in them, haustoria begin to develop from the mycelium in their intercellular spaces. The formative influence of the fungus, therefore, appears to be coincident with the formation of haustoria. The development of haustoria the author associates with the formation of soluble carbohydrates whose presence can be shown in the older but not in the embryonic cells. The presence of hyphae among the embryonic cells shows that the growing point is not protected from invasion by substances toxic to the fungus.

Regarding the general development of the fungus, the author finds that in the rapidly growing shoots the hyphae advance in the tracheae, by means of which the fungus is enabled to keep pace with the growth of the plant. From the tracheae the infecting hyphae enter the parenchymatous tissue of the cortex, pith, and leaves. The cambium, like the embryonic cells of the growing point, is not infected. The formative influence of the mycelium on

<sup>3</sup> TISCHLER, G., Untersuchungen über die Beeinflussung der *Euphorbia Cyparissias* durch *Uromyces Pisi*. *Flora* 104:1-64. figs. 26. 1911.

the stems is slight, but in the leaves the cells undergo more active divisions than those of normal leaves, and the intercellular system is more developed. These changes seem to be characteristic of the fungus, but most of the other morphological changes associated with it can also be induced by other conditions. Both in the stem and in the leaves the localization of the mycelium is dependent upon the presence of soluble carbohydrates in the tissues.

In the later stages of development of the plant, processes of disorganization begin. The death of the leaf cells is accompanied by processes characteristic of cells which are being slowly poisoned. Of the parts of the fungus the haustoria are the most persistent. In the rhizomes they become greatly developed and form a sort of pseudo-parenchymatous tissue in the cells, but they are not the sources of infection in the following year.

Of interest in connection with a consideration of the relations between parasitic fungi and their hosts are the experiments of MORGENTHALER<sup>4</sup> showing that the production of teleutospores is determined more by the state or condition of the host than by the influence of external factors. The author investigated the factors influencing the production of teleutospores of *Uromyces Veratri* on *Veratrum album*. He found that cutting the veins of leaves or wounding the leaves in other ways led to a production of teleutospores in the neighborhood of the wounded tissue, while in the other areas of the leaf uredospores predominated. In general, any cause that affects the leaf unfavorably leads to the production of teleutospores. In standing plants teleutospores are first formed on the lower leaves because these lose their vitality first; but if the plants are cut and kept in water, the upper leaves wilt and become discolored first. In that case teleutospores appear on the upper leaves first, even if all have been inoculated at the same time. From a number of such experiments the author concludes that the production of teleutospores is determined by the changes leading to the withering or dying of the infected parts of the host plants. This conclusion is further strengthened by a number of observations on the distribution of uredospores and teleutospores in relation to the state of the infected parts of the host in herbarium material.

KUSANO<sup>5</sup> gives an account of chloranthic deformation of the flowers of *Prunus Mume* caused by the mycelium of *Caeoma Makinoi*. As a result of the action of the mycelium of this fungus on the primordia of the floral parts, the course of their development is changed so that leaflike structures are produced in place of floral organs. The degree of transformation differs in different flowers. In extreme cases the cuplike receptacle bears a tuft of well developed leaves which in no way resemble floral organs. In other cases leaves take the place of only one or more of the whorls of floral organs, or some of the

<sup>4</sup> MORGENTHALER, O., Über die Bedingungen der Teleutosporenbildung bei den Uredineen. Centralbl. Bakt. II. 27:73-92. figs. 18. 1910.

<sup>5</sup> KUSANO, S., On the chloranth of *Prunus Mume* caused by *Caeoma Makinoi*. Jour. Coll. Agric. 2:287-326. pls. 17, 18. 1911.

organs are only partly modified. The many resulting types are described in detail by the author. The degree of transformation is correlated with the state of development of the primordia at the time that they are infected. The influence of the fungus evidently does not extend beyond the tissue actually invaded. These conclusions, however, are drawn from observations of the visible transformations. No histological details, which would be exceedingly interesting in this case, are reported.

DIETEL<sup>6</sup> has published a second instalment of his studies on the factors influencing the germination of teleutospores. Among the results reported the following are of interest. The teleutospores of *Melampsora Larici-Tremulae* Kleb. are capable of germinating in March and later. Their germination takes place readily at temperatures between 8° C. and 26° C. In the study of *Puccinia graminis* Pers., it was found that the abnormal mode of germination, sometimes observed in teleutospores of this species, is determined by the temperature. At temperatures below 23° C. normal germination takes place, but at higher temperatures the teleutospores simply produce long germ tubes which occasionally become segmented. A similar mode of germination of the teleutospores of *P. Malvacearum* has been observed by TAUBENHAUS<sup>7</sup> and also by ERICKSSON,<sup>7</sup> who attributes to the "conidia" abjoined by the segmentation of the germ tube a special function in the biology of this rust. DIETEL finds, however, that in *P. Malvacearum* the formation of sporidia, or of germ tubes which segment into "conidia," is determined by the conditions under which germination takes place and not by functional differentiation of the spores. High temperature and lack of moisture favor the production of abnormal germ tubes.

To the few observations which have been made on the transmission, from the stock to the scion, and inversely, of grafted plants, of qualities producing immunity from the attack of fungi to which one or the other is subject, FISCHER<sup>8</sup> adds a further observation supporting the general conclusion that no such mutual influence between the stock and the scion exists. He finds that plants of *Mespilus*, which cannot be infected by the basidiospores of *Gymnosporangium confusum*, remain immune even when united by grafting with susceptible species of *Crataegus*, nor is the susceptibility of the *Crataegus* changed.

A special case is presented by *Crataegomespilus Asneresii*, which is a chimaera consisting of a *Crataegus* core with a *Mespilus* epidermis. Here FISCHER found that the germ tubes of the basidiospores penetrated the epidermis and infected the *Crataegus* tissue underneath. He reserves his opinion, however, as to any mutual influence of the two parts of the plants, stating

<sup>6</sup> DIETEL, P., Versuche über die Keimungsbedingungen der Teleutosporen einiger Uredineen. II. Centralbl. Bakt. II. 35:272-285. 1912.

<sup>7</sup> Rev. in BOT. GAZ. 54:431-433. 1912.

<sup>8</sup> FISCHER, ED., Beiträge zur Biologie der Uredineen. Mycol. Centralbl. 1:195-198. 1912.

that it is not certain that the species of *Mespilus* entering into the foregoing chimaera is immune, and even if it were, the result of the experiment does not imply that the *Mespilus* epidermis had become susceptible, since it is known that germ tubes of fungi frequently penetrate inert membranes and even the epidermis of plants in whose tissues they are unable to make any further growth.

ORTON<sup>9</sup> describes a number of cases of correlation in the distribution of certain heteroecious species of *Puccinia* and *Uromyces*. The forms thus correlated have for their telial hosts the same species or closely related species of the same genus, while their aecidia occur on alternate hosts which are either identical or which are species of one genus. The aecidia and the uredospores of the associated rusts are similar in structure, form, and color, while the teleutospores differ only in number of cells. As examples may be cited *Puccinia subnitens* and *Uromyces Peckeanus*, both of which occur on *Distichlis spicata* and have aecidia similar in their essential characteristics on species of the Chenopodiaceae; also *Puccinia Caricis-Asteris* and *Uromyces perigynius* with teleutospores on species of *Carex* and aecidia on members of the Compositae. This condition appears to point to a close relationship between the two genera *Puccinia* and *Uromyces*.

In opposition to the view that rust-infected grains of cereals are the agencies by which the grain rusts are carried over from year to year, ERIKSSON<sup>10</sup> points out that grains bearing rust pustules are, both in his own experiments and according to statements in the literature, of very rare occurrence; and that plants developing from such grains do not become infected earlier nor more severely than plants from normal seeds. Furthermore, a cytological study of a large number of plants from plots which afterward were badly rusted failed to show the presence of mycelium by means of which the rust might have lived through the winter. He concludes, therefore, that the rust pustules on infected seed grain are of no significance in connection with the rust of the grain crop.—H. HASSELBRING.

**Chloroplasts and chlorophyll.**—LIEBALDT's work<sup>11</sup> on chloroplasts emphasizes again the important part colloidal chemistry is coming to play in physiological problems. The chloroplast is considered a two-phase disperse system. The pigments, especially the green ones, constitute the lipid phase, and the stroma, insoluble in lipid solvents, coagulable with heat and alcohol, and swelling in water, is the hydroid phase. The lipid phase shows amicrotic (in particles beyond the vision of the ultramicroscope) dispersal through the

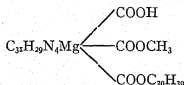
<sup>9</sup> ORTON, C. R., Correlation between certain species of *Puccinia* and *Uromyces*. *Mycologia* 4:194-204. pls. 2. 1912.

<sup>10</sup> ERIKSSON, J., Rostige Getreidekörner- und die Überwinterung der Pilzspecies. *Centralbl. Bakt.* II. 32:453-459. 1912.

<sup>11</sup> LIEBALDT, ERNA, Über die Wirkung wässeriger Lösung oberflächenaktiver Substanzen auf die Chlorophyllkörner. *Zeitsch. Bot.* 5:105-113. 1913.

hydroid phase, hence the chloroplast is generally homogeneous when viewed with either the microscope or the ultramicroscope. The hydroid phase of the chloroplast absorbs considerable additional water when it is brought into direct contact with this agent. This disturbs the dispersion of the two phases and the green pigment accumulates in various regions, giving the plastid a granular appearance. This, the writer believes, is not a complete separation of the two phases, for the green granules are not as dark colored as the separate drops of the pigment, and the lipoid phase will not strain with Sudan III nor the hydroid phase with neutral red, both of which occurs in a true separation of the two phases. Water solutions of various alcohols in concentrations too low to coagulate the hydroid phase hasten and accentuate the deformation caused by distilled water, but do not cause a true separation of the two phases. If the alcohol is sufficiently concentrated to coagulate the hydroid, a complete separation occurs. In this process the separation of the pigment passes from amiconic dispersal through submicronic (particles visible to the ultramicroscope but not to the microscope) and micronic (visible to the microscope) to complete separation. This work gives quite a different picture of the relation existing between stroma and pigments in the normal chloroplast from that generally depicted in texts. The texts generally speak of the pigment being held in the meshes of the spongy stroma or aggregated in the outer layers of the plastid. The author can gain either of these pictures by one or another method of deformation. He does not deny that such a deformation occasionally occurs even in the living active cell, but the chloroplast is generally homogeneous and shows amiconic distribution of the two-phase system. This agrees with the dispersal of the various phases in the protoplasm which is optically empty aside from the microns in it. Plastids of the various plant groups vary in their consistency and resistance to agents; those of the Florideae are most nearly liquid.

By use of alcohols as solvents, the author was able to obtain crystals of the green pigments from many green plants, ranging from the algae up. The form and structure of the crystals vary with the alcohol used. The advance of our knowledge of the chemistry of chlorophyll during the last half-decade enables us to state with reasonable certainty the general constitution of the green pigments as they exist in the plastid, also of the crystalline products in methyl and ethyl alcohol. Tswett's contention that there are two green pigments (termed by him  $\alpha$  and  $\beta$  chlorophyllin) has been confirmed by WILLSTÄTTER, who has shown that they are different oxidation stages of the same substance. According to this writer one of them can be represented as follows:



The molecule bears three carboxyl groups. One is free, another bears a methyl group, and the third a phytol group. In extraction with ethyl alcohol the

phylol group is displaced by the ethyl group and the ethyl derivatives crystallize out. With methyl alcohol the crystals are the methyl derivatives. WILLSTÄTTER speaks of the native green pigments as phytylchlorophyllids. On the same basis the ethyl derivatives are ethylchlorophyllids. The displacement of the phytol by the ethyl group is hastened by an esterase (chlorophyllase). The phytol derivatives are amorphous, while the ethyl and methyl derivatives are crystalline. The constitution of the green crystals given with ketones, esters, and aldehydes is unknown.—WM. CROCKER.

**Silver leaf.**—Güssow<sup>12</sup> reports that the peculiar disease known as "silver leaf" is common on fruit trees in Canada. This disease has been recognized and has received distinctive names in several European countries. Its chief symptom, as its name indicates, is a silvery appearance of the leaves which is brought about by the separation of the epidermis from the palisade cells and the consequent filling of the resulting space with air. The wood of the diseased trees is browned and contains mycelium. After the death of the affected trees, fruit bodies of *Stereum purpureum* develop on the trunks and branches. This fungus has been shown by PERCIVAL, PICKERING, and others to be causally associated with the disease. The observations of these investigators are confirmed by Güssow's experiments in Canada, where in many cases he was able to produce the disease in 100 per cent of the inoculated trees. He also found that scions grafted on diseased trees soon became infected. In all cases the wood is infected with the mycelium of *Stereum purpureum*, but the most striking feature of the disease is the total absence of the mycelium from the diseased leaves. The mode in which the separation of the epidermis from the underlying tissue is brought about by the fungus was not determined. The disease appears to furnish an example of physiological effects wrought by the action of the mycelium on parts of the host not actually invaded. Such phenomena, where the effects cannot be traced to mere mechanical injury, are practically unknown in the field of plant pathology.—H. HASSELBRING.

**Cytology of Laboulbeniales.**—As a sequence to his general introductory account<sup>13</sup> of the cytology of the Laboulbeniales, FAUL<sup>14</sup> has published a full account of the special morphology of two species, *Laboulbenia chaetophora* and *L. Gyrinidarum*. These two forms lack antheridia; therefore the study of them is not complicated by the question of fertilization by spermatia. The young procarp consists at first of the carpogonium, the trichophoric cell, and the trichogyne. The nucleus of the carpogonium and that of the trichophoric cell divide, and at about the same time the wall between the two cells

<sup>12</sup> GÜSSOW, H. T., Der Milchglanz der Obstbäume. Zeitschr. Pflanzenkrank. 22:385-401. figs. 1. pls. 2. 1912.

<sup>13</sup> Rev. in BOT. GAZ. 54:84. 1912.

<sup>14</sup> FAUL, J. H., The cytology of *Laboulbenia chaetophora* and *L. Gyrinidarum*. Ann. Botany 26:325-355. pls. 4. 1912.



disappears, so that the four nuclei come to lie in a single cell. The upper and lower ends of this cell are cut off as the "restored trichophoric cell" and the inferior supporting cell. Each of these contains a single nucleus. The two remaining nuclei undergo a series of conjugate divisions, as a result of which a superior supporting cell, and sometimes a secondary inferior supporting cell, each with two nuclei, are cut off. Two nuclei, presumably one derived from each nucleus of the original pair, remain in the parent cell or ascogonium. The ascogonium may either give rise to asci directly, thus itself becoming an ascogenous cell, or it may divide and give rise to two ascogenous cells. The only nuclear fusion in the life cycle of the plant is that which takes place in the ascus.—H. HASSELBRING.

**Mitochondria.**—The literature on mitochondria is growing, but as it grows the difficulty in defining the structures becomes greater and greater. Just as centrosomes were followed by centrosome-like bodies and blepharoplasts by blepharoplastoids, the mitochondria are now followed by mitochondria-like structures. WOYCICKI<sup>15</sup> describes in the pollen mother cells and microspores of *Malva silvestris* mitochondria-like bodies, which first appear as small granules, then become vacuolate and divide by constriction, and finally disappear completely after the formation of the intine. Starch is entirely lacking during these stages, starch grains first appearing after the exine has become differentiated. The mitochondria-like bodies have nothing to do with leucoplasts or the formation of starch. In *Malva* they resemble somewhat the proteid vacuoles of Coniferales.

Improvements in technic have certainly brought to light some minute structures of the cell which were previously overlooked, but what these structures are and what their significance may be, are problems still awaiting solution.—CHARLES J. CHAMBERLAIN.

**Wilting coefficient in alkali soils.**—In a series of 14 alkali soils, graduated according to their salt content, KEARNEY<sup>16</sup> has shown the wilting coefficients to be practically identical, but the time required for the exhaustion of the water available for growth steadily increased with the increasing salt content. The plants were proportionately smaller when the wilting coefficient was reached in the soils of greater salt content, that is, the presence of alkali increased the quantity of water transpired in producing a unit weight of dry matter. With too great a quantity of salts, pathological conditions were evident in the plants and the wilting coefficient was not reached.—GEO. D. FULLER.

<sup>15</sup> WOYCICKI, Z., Über die mitochondrienähnliche Gebilde in den Gonotokonten und Gonen bei *Malva silvestris* L. Sitzungsber. Warschauer Gesell. Wiss. 5:167-182. pls. 1, 2. 1912.

<sup>16</sup> KEARNEY, THOMAS H., The wilting coefficient for plants in alkali soils. Bur. Pl. Ind. Circ. 109. pp. 9. 1913.

**Morphology of the podocarps.**—SINNOTT<sup>17</sup> has investigated the reproductive structures of the Podocarpaceae and has come to some conclusions in reference to the relationships of the group. The detailed results are too numerous to be cited here, but the author has canvassed the structures of the ovulate strobilus, the characteristic male gametophyte, the wings of the pollen grains, the female gametophyte, fertilization, the proembryo, and the endosperm, and has concluded that the group has been derived from the Abietineae through forms resembling *Podocarpus*, which is therefore the oldest genus. Some striking resemblances between the podocarps and araucarians further suggest that both of these groups may have arisen from an ancient group closely allied to the Abietineae. A connection with the taxads is also suggested by resemblances between *Cephalotaxus* and certain species of *Podocarpus*, the conclusion being that the Taxineae, through *Cephalotaxus* (its most ancient genus), may have arisen from some ancient member of the Podocarpaceae.—J. M. C.

**Adventive branches in *Frullania*.**—MISS LORENZ<sup>18</sup> finds that 4 of the 11 species of *Frullania* in New England reproduce vegetatively by adventitious shoots. From her statements it seems that a marginal cell of a leaf enlarges; that the first two planes of division are anticlinal, giving a quadrant of more or less unequal cells; that the next plane of division is periclinal; that from one of the outer cells of the resulting octant a pyramidal apical cell which gives rise to the shoot is developed. The first leaves of the shoot are rudimentary, but very soon the adult form appears. Ventral leaves are also delayed. As should perhaps be expected, these vegetative shoots are more frequent on dioecious than on autoecious species.—W. J. G. LAND.

**Origin of species in *Hieracium*.**—OSTENFELD<sup>19</sup> has conducted an extensive set of experiments with *Hieracium* to discover the possible relationship between its polymorphism and its strong tendency to apogamy. He has reached the conclusion that new forms arise as hybrids and also by single variations (mutations), and that in both of these cases "the prevailing apogamy supports their existence and constancy." This means that the polymorphism of the genus is correlated with its apogamy.—J. M. C.

<sup>17</sup> SINNOTT, EDMUND W., The morphology of the reproductive structures in the Podocarpaceae. Ann. Botany 27:39-82. figs. p. pls. 5-9. 1913.

<sup>18</sup> LORENZ, ANNIE, Vegetative reproduction in the New England Frullaniace. Bull. Torr. Bot. Club 39:279-284. figs. 3. 1912.

<sup>19</sup> OSTENFELD, C. H., Experiments on the origin of species in the genus *Hieracium* (apogamy and hybridism). New Phytol. 11:347-354. 1912.

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BOTANICAL GAZETTE

SEPTEMBER 1913

## SEMIPERMEABILITY OF SEED COATS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 176

CHARLES A. SHULL

(WITH NINE FIGURES)

## I. Introduction

As our knowledge of the physical and chemical characteristics of seed coats increases, the importance of the physiological rôle and the biological significance of these structures become more and more apparent. Much work has been done upon the germination of seeds without an adequate knowledge of the real conditions offered to the embryos for their development. Many chemical substances and various ethereal stimuli have been used to influence the germination of seeds, and as a rule the seeds have been used with coats intact. This has been true especially of the Germans who belong to the vitalistic school. The assumption has been that the coats of seeds, and cell walls generally, are permeable to practically all water-soluble substances, and that the dead membranes and cell walls do not modify ethereal and chemical stimuli which act through them, simply because they are dead.

Recently a number of papers have appeared which have a very important bearing upon these problems. In 1907 BROWN (10) reported the discovery of a semipermeable membrane forming the outer layer of the seeds of *Hordeum vulgare* var. *coerulescens*, and later (11) published an account of the selective permeability of this outer dead membrane of the seed. This work was followed by

SCHRÖDER (33), who found the same kind of selectively permeable coat in wheat. ATKINS (5) failed to find this character in the coats of beans. He found that the absorption of water from living and dead seeds was identical until germination commenced, at which time the osmotic phenomena of the living cells were manifested. The forces concerned in the initial stages of water intake are, according to ATKINS, those of capillarity and imbibition; but on germination, osmotic pressure begins to influence the amount of water taken up by the living seeds. As SCHRÖDER has pointed out (33, p. 188, footnote), ATKINS failed to take into account the open micropyle of the Leguminosae used; but the same seeds on moist sand, with the micropyle turned up, absorbed 90 per cent of their dry weight from 10 per cent NaCl in 6 days, according to SCHRÖDER. And since the present paper was written, TJEBBES (35) has found that the seeds of the sugar beet probably have a selectively semipermeable membrane as part of the inner seed coat.

Up to the present, therefore, semipermeable membranes have been reported only in the Gramineae and Chenopodiaceae. However, another interesting discovery was made in 1907 by BECQUEREL (7), who showed that the thoroughly dried seed coats of certain plants were impervious to various gases and to such penetrating substances as absolute alcohol, chloroform, and ether. He made no attempt to determine whether these coats were also semipermeable.

During the last two years I have been investigating the character of the seed coat of *Xanthium* with special reference to the work of BECQUEREL, BROWN, and SCHRÖDER, and present here the results of the work. I wish to acknowledge with thanks the encouragement and helpful advice of Dr. WILLIAM CROCKER, and to express my appreciation of the excellent facilities afforded me by the Hull Botanical Laboratory.

## II. Experimentation

### I. MOISTURE AND PERMEABILITY

The discovery by BECQUEREL that various gases, and alcohol, chloroform, and ether would not penetrate certain seed coats if completely dried, seemed so unusual that attempts were made to repeat his experiments, using the testa of *Xanthium glabratum*

cemented over the end of a glass tube fitted with a perforated rubber cork, the tube being then filled with mercury, and set up as a sort of barometer. Although the coats were supported by strong cloth, they were too delicate to withstand the strain, and would always burst on being inverted over the mercury cup. Shorter columns also were tried. In one case a column of mercury 20 cm. in height was sustained for four days without any diffusion of atmospheric gas taking place through the membrane.

Several attempts were made to test the diffusion of oxygen through the dry seed coat by means of an apparatus like that used by CROCKER (14) to test the diffusion through moist coats. These tests ran for only a few hours at a time and the results were negative. No measurable diffusion was noted, but the slightest injury to the coat was found to permit a rapid passage of oxygen.

Much better results were obtained with the experiments on the permeability of dry coats to ether, chloroform, acetone, and absolute alcohol. Seeds of *Xanthium glabratum* were dried for two weeks at 40° C., then put into a desiccator over phosphoric anhydride at 10 mm. atmospheric pressure for 18 days at 40° C., after which they were stored in a similar desiccator for use.

Water-free ether was prepared by treating the best ether obtainable with alcohol-washed KOH in sticks for 12 hours, and then distilling it with ribboned metallic sodium in both distilling and receiving flask. The sodium was allowed to act in the receiving flask until every trace of water had disappeared.

Five lots of dry seeds were then immersed in dry ether, and kept at a constant temperature in a Freas thermostat for various lengths of time from 2 to 36 days. On removing them from the ether the seeds were carefully dried, soaked in water, the testas removed, and the embryos placed in germinative conditions; 87 per cent of the seeds germinated, and the young plants were just as vigorous as the untreated controls. In those sets of seeds soaked for 16 and 36 days respectively, 100 per cent of both uppers and lowers germinated, and the average growth in length in 6 days was for the uppers 9.5 cm., for the lowers 11 cm.

Seeds carefully dried were also placed in chloroform, absolute alcohol, and acetone without the precaution of drying the fluids.

In all cases the dried seeds remained for many days (16-30) in the fluids without showing any injury, as shown by their normal germination afterward. That these fluids do not penetrate the testa can be demonstrated indirectly by using seeds with defective coats. In no instance were such seeds found to be viable after more than about 15 hours' immersion in absolute alcohol, ether, chloroform, or acetone. The length of time seeds with broken coats can remain alive in these substances depends upon the size and position of the defect. If the break occurs immediately over the hypocotyl, a few hours' immersion will kill the seeds; but if at the tips of the cotyledons, they will remain viable some hours longer.

The question naturally arises as to how much moisture can be put into these liquids, and still leave the seeds uninjured by long soaking. Commercial 95 per cent alcohol was found not to kill merely air-dried seeds in 4 days' time, but had killed them within 8 days. A series was then run in 90, 80, 70, 50, and 35 per cent alcohol. In a few hours the seeds in the lowest three grades had become excessively swollen and semitranslucent; they were found to be dead. Those in 80 per cent were by no means so swollen, but had been killed. Those in 90 per cent were apparently hard and sound, but at the end of 3 days all were dead. They may have been killed in considerably shorter time than this, as they were not tested for germination till the end of 3 days.

The results of these experiments confirm the findings of BECQUEREL, who kept various seeds in alcohol, ether, and chloroform for a full year without any serious loss of viability. While these facts seem remarkable, they merely confirm the results reported a good many years ago by GIGLIOLI (21), who obtained, for instance, the germination of 20 per cent of alfalfa seeds after more than 16 years in a saturated solution of  $\text{HgCl}_2$  in absolute alcohol. GIGLIOLI says that many of these seeds produced plants which flowered and fruited normally after this long exposure to destructive gases and liquids. The protective nature of the dry seed coats of certain seeds, therefore, is fully established, and is due to impermeability.

The results of these experiments offer no evidence against BECQUEREL's view that gases, especially oxygen, are not able to

diffuse through the thoroughly dried seed coats of certain seeds. CROCKER (14) was inclined to believe, from his study of the respiratory ratio in *Xanthium* seeds, that more oxygen diffused through the dry seed coats than through those which had not been thoroughly dried. This conclusion is probably shown now to be incorrect. The gas exchange noted by him in the case of dried coats may have occurred at least partially through defects in the coats; for, as I shall show later, there are a good many seeds which prove to have defects which are invisible, even on microscopic examination, and these defects may possibly be increased in number by the process of drying.

## 2. SELECTIVE SEMIPERMEABILITY OF XANTHIUM SEED COATS

A few preliminary tests to determine the amount and rate of imbibition of *Xanthium* seeds in solutions of sodium chloride of various strengths indicated the existence of a very efficient semipermeable membrane in the seed coat. Water enters the seed very rapidly under the powerful forces of capillarity and imbibition until more than 50 per cent of the air-dry weight of the seeds has been taken up. Nearly all of the water is taken up in 12-15 hours, as shown by the curves in figs. 1 and 2.

This rapid intake of water is in sharp contrast with that which occurs in the Gramineae. In the seeds of the latter the rate of water intake is considerably slower, and extends over 7 or 8 days or even longer, before saturation is reached. Evidently the protective structures influence the rate at which water passes into the seeds.

When strong salt solutions instead of distilled water are used with *Xanthium* seeds, the powerful internal forces, capillarity, imbibition, and perhaps the chemical

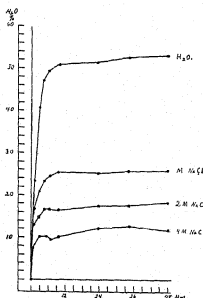


FIG. 1.—Curves of imbibition for seeds of *Xanthium* in salt solutions of various strengths, and in water; time element plotted on the axis of abscissae, percentage increase over air-dry weight on the axis of ordinates.

attraction of colloids, cause the water to enter with almost as great initial rapidity as when pure water is used, until the osmotic pressure of the salt on the outside balances the internal forces mentioned; then the entrance of water quickly ceases. After equilibrium is established in any given salt solution, increasing or decreasing the density of the solution extracts water from the seed or permits water to enter it in accordance with the direction of the disturbance. The adjustment continues rapidly after the disturbance until the equilibrium is reestablished, when the amount again remains constant.

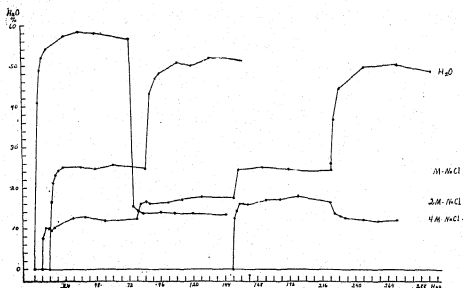


FIG. 2.—Curves showing entrance and withdrawal of water in *Xanthium* seeds on shifting from water to salt solutions, or vice versa, at three-day intervals; the semipermeability of the membrane is well illustrated by the behavior recorded in these curves; curves 2, 3, and 4 are displaced to the right to avoid confusion.

The speed with which this adjustment of internal and external forces takes place in *Xanthium* is again in contrast with the rate of adjustment in grasses, such as wheat and barley, in which the rate of adjustment is slow, requiring days rather than hours to establish equilibrium. It appears that the coat in *Xanthium* offers very slight resistance to the passage of water, while in the Gramineae a comparatively much greater resistance is offered by the coat structures.

This relation of osmotic pressure to capillary and imbibition forces, chemical and physical, through the semipermeable coat of the *Xanthium* seed, is splendidly illustrated by figs. 1 and 2, which



show the imbibition curves for distilled water, and for molecular, two-molecular, and four-molecular solutions of NaCl as the seeds were shifted from one strength to another at three-day intervals. The close agreement of the constant portion of the curves in each strength of solution should be noted. The character of the curve during the last two days of each three-day interval indicates that the salt does not enter in appreciable amounts. Comparison with the type of curve given by SCHRÖDER (33, p. 189) for wheat shows that this membrane is at least as efficient in excluding NaCl as the coats of the Gramineae.

By appropriate chemical methods it has been shown that no passage of the salt through the membrane occurs. It was necessary, however, to test seeds singly, since one finds rather frequently seeds which have invisible defects in the coat which allow the passage of salts.

Many other chemical substances, usually in molecular solutions, were used to determine the range of selective semipermeability. The permeability or non-permeability to each substance was judged by the amount of imbibition water taken up from such solutions of acids, bases, and salts. Anyone who investigates the subject will be convinced that this means of determining semipermeability is sufficiently accurate for all ordinary purposes. The results, in percentage of increase over air-dry weight by imbibition, are given in table I.

From this table it is seen that the nitrates as a class, and especially silver nitrate, penetrate the coats. Iron sulphate slowly enters, while copper sulphate does not. The penetration of copper sulphate can be detected, if it occurs, by removal of the testa and examination of the embryo. Occasionally as high as 20-40 per cent of the seeds show slight localized penetration of  $\text{CuSO}_4$  through defects in the coat too slight to be seen on microscopic examination of the dry seeds. Because of these defects the figures given for any salt are approximations only; but in case there is general permeability it is easily recognized by the amount of imbibition. Since 60-80 per cent of the seeds show no penetration even after prolonged soaking in  $\text{CuSO}_4$ , and since they retain their vitality perfectly, notwithstanding the very highly poisonous character of

the solution, it seems reasonably certain that copper sulphate does not penetrate a sound testa.

TABLE I  
PERCENTAGE OF IMBIBITION  
(BASED ON AIR-DRY WEIGHT)

OSMOTIC PRESSURE (CALCULATED FROM THE LANDOLT- BÖRNSTEIN TABLES)* IN ATMOSPHERES	SUBSTANCE	END OF THREE DAYS		TEN DAYS	REMARKS
		Exp. I	Exp. II	Exp. II	
	Water	54.94	53.95	.....	End of two days
38.02.....	M-NaCl	26.	24.46	.....	
72.01.....	2M-NaCl	21.58	18.47	.....	
130.62.....	4M-NaCl	12.16	13.44	.....	
	Sat. NaCl	7.12	6.2	.....	
35.53.....	M-AgNO <sub>3</sub>	49.83	46.57	70.89	
37.66.....	M-NH <sub>4</sub> NO <sub>3</sub>	32.47	33.	40.6	
37.67.....	M-NaNO <sub>3</sub>	34.18	28.88	32.09	
36.53.....	M-KNO <sub>3</sub>	37.89	38.87	41.29	
28.25.....	M-CuSO <sub>4</sub>	34.48	35.4	35.59	
	M-FeSO <sub>4</sub>	34.39	37.48	44.73	
39.6.....	M-KCl	35.15	37.62	38.3	
	M-K <sub>2</sub> CrO <sub>4</sub>	24.55	32.04	36.91	
	M-Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	28.37	.....	.....	
	5% HgCl <sub>2</sub>	70.2	.....	.....	
	CH <sub>3</sub> OH	66.6	.....	.....	10 volume per cent
	C <sub>2</sub> H <sub>5</sub> OH	57.14	.....	.....	" " " "
	C <sub>3</sub> H <sub>7</sub> OH	69.25	.....	.....	" " " "
	Glycerol	29.32	.....	.....	" " " "
	Ether sat.	53.95	.....	.....	End of 5 hrs.
	Iodine, 5 per cent in KI.....	.....	.....	.....	Penetrates
	M-Sucrose	34.41	.....	.....	In 23 hrs.
	M-Fructose	35.54	.....	.....	
	M-Glucose	36.51	.....	.....	
	M-Lactose	35.47	.....	.....	
	M-KOH	79.	.....	.....	In 35 hrs.
	M/10 KOH	72.2	.....	.....	
	M-NaOH	102.06	.....	.....	In 10 hrs.
	M/10 NaOH	50.2	.....	.....	In 48 hrs.
	M-HCl	32.87	.....	.....	
	M-H <sub>2</sub> SO <sub>4</sub>	42.06	.....	.....	
	M-HNO <sub>3</sub>	73.33	.....	.....	
	M-Tartaric	36.87	.....	.....	In 11 hrs.
	M-Acetic	57.3	.....	.....	
	M-Lactic	55.84	.....	.....	
	M-Citric	37.06	.....	.....	

\*In these calculations from the Landolt-Börnstein tables correction is made only for dissociation. The osmotic pressure of 2M and 4M-NaCl is probably somewhat higher than here indicated, due to the formation of hydration compounds. Saturated NaCl is believed to exert 375 atmospheres of osmotic pressure.

Mercury bichloride enters the seed rapidly, as do also aqueous solutions of iodine in KI, the monatomic alcohols, ether, the alkalis,

and most of the acids, except perhaps hydrochloric and tartaric. The entrance of sulphuric is very slow. The triatomic alcohol, glycerol, does not enter, nor do the sugars.

The semipermeability is not dependent on any living cells in the membrane, but is purely a physical phenomenon. Boiling the seeds does not destroy this character of the coat, nor do such poisons as  $\text{HgCl}_2$ ,  $\text{AgNO}_3$ , iodine, the alcohols, ether, etc. Solutions of sodium chloride extract water rapidly from seeds which have imbibed almost 70 per cent of their weight in 5 per cent  $\text{HgCl}_2$ . The peculiarity of the testa must therefore depend upon the physical structure and chemical composition of the dead cell walls, and upon the relations it may assume toward the various solutes and solvents, not upon any vital conditions.

The seed coat of *Xanthium* possesses a great advantage over the membranes discovered by BROWN and SCHRÖDER in barley and wheat as an object of research; for in *Xanthium* the entire coat is easily removed after soaking for a few hours in water, and can then be used readily as an osmotic membrane.

For this purpose I have used the apparatus illustrated in fig. 3. A glass tube about 6 cm. in length and 1.5 cm. in diameter is closed at both ends with short rubber corks, each perforated by a hole 3 mm. in diameter. Since the corks must fit tightly into the glass tube, the perforation of one of the corks is prevented from collapsing by inserting a short piece of glass tubing 3 mm. in diameter. The testa is prepared for use by cutting it longitudinally along one side, and cutting off the ends. The testa can then be opened out and dried under a weight, thus producing a rectangular membrane about  $6 \times 8$  mm.

This membrane is cemented carefully to the rubber cork which has been prevented from collapsing by the narrow glass tubing. It is best to place a thin layer of wax over the cork first, then press the membrane firmly into the wax, after which the edges of the dry



FIG. 3.—Osmotic apparatus used in testing directly the semipermeability of *Xanthium* seed coats; description in text.

membrane are carefully sealed with more of the wax, leaving only that portion of the membrane immediately over the hole in the cork entirely free from wax. In my experiments the area of membrane left uncovered has been approximately equal to the area of the perforation through the cork.

The 6 cm. glass tube is now placed in vertical position, with the membrane at the lower end. A 4 M solution of NaCl is introduced through the upper cork until the chamber is quite filled. Care must be taken that small bubbles of air which tend to remain in the perforation of the lower cork while the chamber is being filled do not prevent the solution from filling this perforation. Otherwise the membrane will not be in contact with both water and solution.

Finally a long glass tube 3 mm. in diameter is carefully inserted through the upper cork. The salt solution will rise a short distance in the narrow tube, due to displacement. After making sure that the apparatus is entirely free from salt solution externally, the chamber is suspended in a vessel of distilled water with the liquids at the same level, just as if it were a thistle tube. This apparatus and the experiment itself are so simple that they should be of considerable pedagogical interest, as demonstrating directly the independence of semipermeability of this kind from any vital matter.

In one such experiment a rise of 175 mm. occurred in the narrow tube in 7 days. This is a fairly rapid rise, inasmuch as the tube in which the rise was measured had practically the same cross-section area as the diffusion membrane. It means that on the average a column of water 25 mm. long passed through the membrane in a day's time. The rate of passage is so rapid as to indicate a very slight resistance of the membrane to the passage of water, a condition quite different from that found in the Gramineae, as already suggested.

Here again the impermeability of the membrane was demonstrated by dropping some of the liquid taken from immediately below the diffusion membrane into  $\text{AgNO}_3$ . No visible precipitate was obtained, even at the end of 7 days.

### 3. STRUCTURE AND COMPOSITION OF THE TESTA

The morphological development of the seed coat of *Xanthium* has not been fully traced, but it is probable that the seed coat is

formed in the same way as in another of the Compositae, *Helianthus*, the structure of which has been reported by BRANDZA (9).

In *Helianthus* the inner layer of the 3-layered seed coat is formed from the nucellar epidermis, and is composed of just one layer of cutinized cells. *Xanthium* likewise has a 3-layered testa, the inner layer of which is a single layer of cells excepting over the hypocotyl, where, as CROCKER has shown, it is several cells thick. It is probable that this inner layer, with the exception of its chalazal portion, which lies immediately over the hypocotyl, is derived from the epidermis of the nucellus.

At first it was suspected that the inner stratum might be the semipermeable layer, although CROCKER had suggested in 1906 that the thicker middle one probably excluded oxygen. While attempt was being made to locate the seat of the semipermeability it was discovered that certain substances, the higher alcohols, acetic acid, and the alkalies, occasionally cause a bursting of the middle layer without injuring the single-celled inner lamina. The outer layer, of course, is too loose and chaffy to function osmotically.

In the case of M and M/10 solutions of KOH and NaOH, the bursting of the middle layer is followed by a periclinal separation of the inner layer from the middle one, which is probably the ovular integument, so that the embryo, surrounded by the intact, thin, delicate, one-celled inner layer can be removed from the external coats. This discovery was a very important aid in studying the semipermeability of the different layers of the testa, and illustrates the advantage which removable coats have over such as have been found in the grains. In the barley and wheat all such investigations had to be indirect, because the membrane could not be removed or separated into distinct layers.

The thin inner coat was shown to be a very efficient semipermeable membrane by treating the much swollen seeds surrounded by this membrane with 4M-NaCl. At the time one such seed was introduced into the salt solution it weighed 110 mg. After one hour the membrane was completely collapsed and pressed very tightly against the embryo. It weighed 58 mg. In 4 hours it weighed 52 mg., in 10 hours 51 mg., after which a rise in weight gradually occurred, probably due to a very slow entrance of NaCl.

In 2 days its weight was 59 mg. Fig. 4 shows the curve for loss of water from such a seed which weighed 198.5 mg. at the time it was put into NaCl.

The middle layer, which is several cells thick, was subjected to a direct test by using it as an osmotic membrane cemented over a rubber cork. Its osmotic character is demonstrated by the following figures:

Rise	Time	Rise	Time
45 mm.....	5 hrs.	159 mm.....	32 hrs.
72 " .....	9 "	169 " .....	48 "
125 " .....	20 "	171 " .....	72 "

However, this layer is by no means as efficient as the inner layer. The NaCl could be seen streaming through the membrane into the distilled water, causing the same appearance as the rapid solution of a salt in water.

Neither the middle nor inner membrane is as perfectly semipermeable alone as are both together in the natural condition. Microchemical study of the coat, using NaCl and AgNO<sub>3</sub>, had given evidence in the same direction, that the middle layer allowed salts to reach the inner layer, but that the precipitate of AgCl was not certainly present within the inner layer. The extreme stretching of the coats preceding the bursting, or a physical or chemical modification due to the macerating agent, may be responsible for the impaired efficiency of the separated membranes.

The cellular arrangement in the inner membrane is shown in fig. 5. Microchemical tests showed the walls of this layer to be largely cellulose, with little or no suberization. A dark blue color was obtained on treatment with sulphuric acid and iodine, and the materials of which it is composed are almost completely dissolved in Schweizer's reagent. Practically no coloration was obtained with alkanin and other suberin reagents, except in the cell contents, and these are known not to be respon-

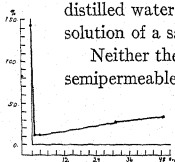


FIG. 4.—Curve showing loss of water by a seed which had become excessively swollen in M-NaOH when later put into 4M-NaCl; the outer coats of the testa had burst, leaving the inner coat, one cell thick, intact; the curve demonstrates the semipermeable character of the inner layer.

sible for this physical character of the membrane, which remains unchanged when the cell contents have been removed by dissolution.

REICHARD (29) some years ago showed that the coats of *Hordeum* are full of tannin, and has now (30) raised the question whether the tannin present there has any effect on the semipermeability of its membranes. The colloidal condition of the tannin, and its known peculiarities chemically, suggested that it might be in a large measure responsible for the physical properties of the coat. He investigated the position of the tannin by microchemical methods, and found that in sections the barley grains showed a sharply defined layer of tannin which could easily form a continuous coat. Treatment of seeds with tannin solvents showed that with such treatment the layer of tannin becomes broader and more diffused. Alcohol (96 per cent) used alone does not dissolve tannin. Following the alcohol treatment with iron sulphate solution as a tannin stain shows that the tannin layer becomes if anything more sharply defined in alcohol of high grade. But if the seeds are soaked first in water, then the tannin shows marked solution effects when subsequently treated with alcohol. Alcohol seems to dissolve tannin only when it has been previously wet.

According to REICHARD's view, the entrance of substances into the seed would depend upon their ability to dissolve the tannin layer, or to dissolve in it. Since strong alcohol does not dissolve tannin, seeds with such a tannin layer might lie in absolute alcohol indefinitely without loss of vitality. But the presence of sufficient moisture to dissolve or partially dissolve the tannin would allow rapid entrance of alcohol, and a consequent killing of the embryo. The bearing of these facts upon the resistance of dry seeds to organic solvents discussed in an earlier section of this paper is obvious.

Hydrates, especially NaOH, have a powerful solvent effect upon tannin; even in M/10 solutions the solvent action is apparent, and

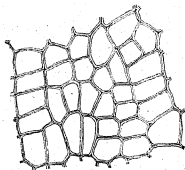


FIG. 5.—Cellular arrangement of a portion of the inner layer of the testa.—Camera lucida, 4 mm. obj., 4 ocular, 160 mm. tube.

as the concentration increases, the action is very rapid. On the other hand, acids do not dissolve tannins, but rather precipitate them from solution, according to REICHARD.

The barley grains used by REICHARD did not seem to be as perfectly semipermeable as those used by BROWN. This variation in semipermeability is attributed to differences in the tannin content from differences in the ripening process. He believes that the tannin is an "assimilation" product of chlorophyll, and that in unripe seeds the tannin has not been fully deposited in the seed coats. Or one can assume that the tannin for some unknown reason has failed to reach the proper chemico-physical condition for complete semipermeability.

While it seems entirely possible that the resistance of dry seeds to alcohol and other reagents such as ether, chloroform, etc., might be related in certain cases to tannin in the seed coats, it does not seem probable to me that semipermeability of these membranes can be explained on these grounds.

There is a considerable amount of tannin in the testa of *Xanthium*, as shown by treatment with ferric chloride, ferric sulphate, potassium dichromate, etc. But the tannin in this case does not seem to form so definite a layer as REICHARD reports for *Hordeum*. It seems to be scattered through the tissue in minute granules, occurring especially in the outer and middle layers of the coat with only very minute quantities in the inner layer. As has been shown, treatment of the coats with molecular NaOH, which is a strong tannin solvent, according to REICHARD, does not destroy its semipermeable character. For these reasons it is the writer's opinion that the property belongs to the cellulose, rather than to substances associated with it in the coat. Further microchemical studies upon the seed coat of *Xanthium* are in progress, and in time it may be possible to analyze the conditions which cause the exclusion of certain salts and the passage of others through the semipermeable coat of seeds.

#### 4. SEMIPERMEABILITY A WIDESPREAD PHENOMENON

In addition to the work on *Xanthium*, a number of seeds in various families have been tested. The results show that the



lifeless membranes forming the coats of seeds frequently exhibit osmotic effects. A number of widely separated families are represented in the list now known to possess such properties: Alismaceae (*Alisma Plantago-aquatica*), Gramineae (barley, wheat, oats, etc.; probably most grasses), Chenopodiaceae (sugar beet), Rosaceae (peach, apple), Leguminosae (*Vicia Faba*, scarlet runner, lima bean), Compositae (*Xanthium*, *Helianthus annuus*). It should be said at once that these seeds do not all exhibit the same degree of semipermeability. The coat of *Xanthium* apparently permits no passage or only very slow passage of certain solutes, comparing favorably with the coats of *Hordeum* and *Triticum* in this respect. Coats of the sunflower and peach are only less efficient, while the bean coats usually allow a noticeable passage of salts. The Leguminosae seem to be less uniform in their behavior than any seeds tested. Certain specimens have very good semipermeable membranes, others have rather poor ones.

As pointed out in the introduction, ATKINS found Leguminosae not to possess semipermeable testas, but had overlooked the open micropyles. Direct methods of testing proved that these membranes do act osmotically. It was not found practicable to wax these membranes onto rubber corks, because the great amount of expansion of the membrane on soaking, after the apparatus was arranged, always resulted in breaking the coat loose from the wax. To overcome this difficulty, the wet bean coats were placed tightly between two perforated rubber corks which were smeared slightly with vaseline, and used just as in the case of *Xanthium* already described. In this case the hole in the cork below the membrane must also be carefully filled with water to insure contact of both fluids with the membrane.

Coats of *Vicia Faba* used with saturated NaCl solution gave a rise of 72 mm. in 36 hours; and of scarlet runner a rise of 135 mm. in 10 days, the rise continuing throughout this time, but amounting to only 17 mm. during the last 4 days. The escape of the salt through the coats of both these legumes was readily demonstrated by use of  $\text{AgNO}_3$ .

It is a pleasure to record the fact that Professor STEVENS of the University of Kansas has for more than 8 years been using the coats

of the lima bean to demonstrate osmosis in his elementary classes in plant physiology. The coats are merely tied over the ends of glass tubes. His experience has been that the coats are irregular in their behavior, and that successful use demands the testing of the membranes, and the choice of those whose behavior with a definite strength of solution is uniform. Thus chosen, the membranes may be used to demonstrate admirably PFEFFER's discovery that osmotic pressure varies directly with the concentration of the solution.

Recently LAVISON (24) studied the entrance of salts into the roots of plants and showed that the cellulose "frames" (*cadres*) around the cells of the endodermis are impermeable to certain salts, and that entering salts must therefore pass through protoplasm in penetrating the root beyond the endodermis. Later (25) he made the further observation that the cellulose cell walls forming the periclinal walls of the endodermis behave toward entering salts like the protoplasm itself. That is, those salts which are excluded by the protoplasm are excluded by the walls also. LAVISON does not say that the walls are selectively semipermeable, but that is seemingly the only possible interpretation of his observation. It should be noted that this applies only to the *young* cellulose walls.

All of this evidence points to semipermeability as a widespread phenomenon among lifeless plant membranes. Of course, the membrane must be permeable to water if it is to be osmotically active. The thin skin of potatoes is impermeable to water solutions, allowing neither salt nor water to pass through. The possibly semipermeable character of cellulose membranes cannot be overlooked in future investigations dealing with the entrance of salts into plant tissues.

#### 5. OSMOTIC PRESSURE AND IMBIBITION FORCES

The experiments upon *Xanthium* seeds with concentrated salt solutions suggested the possibility of measuring the capillary and imbibition forces of seeds by use of solutions of very high known osmotic pressure, since the balancing of osmotic pressure on the one hand against capillarity and imbibition on the other through a perfect semipermeable membrane is a very simple matter.

For this purpose saturated solutions of lithium chloride, which

gives the highest osmotic pressure of any known neutral salt, was employed. The imbibition curve for air-dry seeds known to contain 8-9 per cent of moisture is shown in fig. 6. In the case of both lower and upper seeds there was found a slight loss in weight during the first few hours of soaking in this concentrated LiCl solution. The loss is very small in amount, averaging 0.5 mg. per seed in all the tests made. But after the seeds have been in the solution for 6 or 7 hours, there is a very slow increase in weight, so that at the end of 46 hours the weight of the soaked seeds was the same as when first put into the fluid. The exact significance of this behavior is not very clear, but it is obvious that these seeds do not possess internal forces of sufficient magnitude to withdraw any water from the LiCl solution.

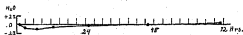


FIG. 6.—Imbibition curve for air-dry seeds in saturated LiCl solution.

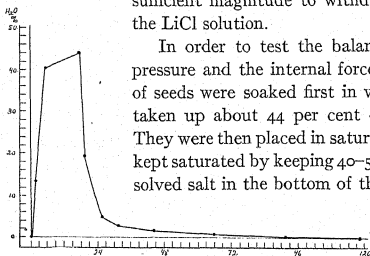


FIG. 7.—Curve showing loss of water from seeds soaked in water before transferring to saturated LiCl solution; imbibition force of air-dry seeds and osmotic pressure of saturated LiCl solution approximately equal.

water had been withdrawn, and in 7 hours the seeds were only 2.5 per cent above their air-dry weight. The water was withdrawn more and more slowly until at the end of 100 hours they reached their original dry weight. The results shown in figs. 6 and 7 indicate that capillarity and imbibition force in an air-dry seed of *Xanthium*, that is, one with 8-9 per cent of moisture, is approximately equal to the osmotic pressure of a saturated solution of LiCl.

it up frequently. The imbibition curve is shown in fig. 7. At the end of one hour, more than three-fourths of the

One of the most difficult problems was to secure adequate data as to the size of the pressures and forces indicated by the results. The literature aside from the work of the physical chemists contains serious disagreements, and the physical chemists have done very little on the osmotic pressure of concentrated solutions. Moreover, there is wide divergence in the interpretation of the little they have done. As the writer is not a physical chemist he has been at a distinct disadvantage in the attempt to clarify a very confusing situation.

In his work on *Hordeum*, BROWN ascribes to a saturated solution of NaCl an osmotic pressure of 125 atmospheres. A few years earlier, RACIBORSKI (28), who investigated the upper limits of osmotic pressure in living plant cells, cites DIETERICI as authority for the statement that saturated NaCl has at 20° C. an osmotic pressure of 375 atmospheres. RACIBORSKI gives the pressure as 349.11 atmospheres at 0° C., the saturation concentration being 35.51 per cent. The figures given by BROWN are apparently based on the assumption that the law announced by VAN'T HOFF in 1887, that osmotic pressure is proportional to concentration, a law based on PFEFFER's work a decade earlier, applies to all concentrations whatsoever, without any allowance for electrolytic dissociation or hydration, both of which must play an important rôle in the pressure of these concentrated solutions. On such an assumption the pressure should be near 125 atmospheres.

That this figure is too low can readily be determined from data as to the electrical conductivity of NaCl derived from the Landolt-Börnstein tables. A 4 M solution would exceed 130 atmospheres, so that a saturated solution should run to 160 atmospheres or beyond, merely due to ionization.

Moreover, there is reason for believing that this law of the relation of osmotic pressure to concentration holds only for lower concentrations, and that still further corrections are necessary in the case of highly concentrated solutions; for the actually observed pressures depart widely from the theoretical requirements as concentration increases. BERKELEY and HARTLEY (8) measured the osmotic pressure of solutions of sucrose, dextrose, and galactose by an ingenious method, and have shown that dilute solutions give

pressures agreeing with the gas laws. But curves representing the pressures in higher concentrations show that the osmotic pressure increases in strong solutions much more rapidly than the molecular concentration.

Formerly it was customary to calculate the osmotic pressure on the basis of solution volumes. MORSE, FRASER, and others, however, found a better agreement between increase of concentration and osmotic pressure when the concentration is referred to a liter of solvent rather than to a liter of solution. RENNER (31) has recently given an excellent summary of the literature on the calculation of osmotic pressures, and holds that this change from liter of solution to liter of solvent will bring into agreement the pressures obtained by plasmolytic and those obtained by cryoscopic methods, the former having been in error.

Although making this change brings agreement between theoretical and observed pressures for more concentrated solutions than was true with the old method of making up molecular solutions, the observed pressures are still considerably above the pressures demanded by our theories, after all these corrections and improvements in method have been made.

The relation of the actual pressures found by BERKELEY and HARTLEY to the pressures demanded theoretically by both methods of calculating pressure from concentration is shown graphically by PHILIP (27, p. 53) by means of curves. RENNER also shows a similar diagram in his recent paper but does not mention PHILIP's discussion. There is little doubt of the correctness of the pressures found by the direct methods of measurement employed by BERKELEY and HARTLEY, as their results have been confirmed recently by TROUTON (36), who uses an entirely new method for the direct measurement. Ether will not take up as much water from a solution as it will from pure water. He determines the pressure necessary to force as much water from a solution of sucrose into ether as the ether will take up normally from pure water (1.05 per cent). The pressure required to force the water content of ether up to 1.05 per cent from a solution of sucrose containing 600 grams per liter was 80 atmospheres. BERKELEY's result was 81 atmospheres with the same strength of sucrose solution, an agreement well within the limits of error.

The findings of BERKELEY and HARTLEY were discussed by CALLENDAR (13), who offers a plausible explanation for the high pressures observed. He suggests that the solute probably enters into a molecular complex with the solvent, forming hydration compounds. This chemical combination of solute with solvent reduces the number of free molecules of water in the solvent, decreases therefore the vapor tension, or, what amounts to the same thing, increases the osmotic pressure; for, as CALLENDAR says: "There is a definite and simple relation between vapor pressure and osmotic pressure which has been verified by LORD BERKELEY and HARTLEY for strong solutions."

The degree of hydration is represented by  $a$  in CALLENDAR's discussion, and he believes  $a=5$  in the case of the sucrose solution used by BERKELEY; and in the case of KAHLENBERG's (23) observations on the rise of boiling-points in concentrated solutions of NaCl, the curve agrees with a hydration value of  $a=6$  up to a concentration in which there are 6 molecules of NaCl present for every 100 molecules of water (CALLENDAR 13, p. 493, diagram p. 492).

CALLENDAR assumes that the molecular complex formed by the hydration is a *definite* chemical compound at any given concentration. In this he does not agree with JONES and BASSETT (22), who claim to have shown that such hydration compounds are indefinite, and may vary from a few to many molecules of water to each molecule of solute in any given concentrated solution.

If the compounds formed were indefinite, it would not be possible to explain the increased osmotic pressure of strong solutions by hydration; but CALLENDAR points out that the determinations of JONES and his co-workers are erroneous at various places, and their conclusions untrustworthy. The writer can only express it as his opinion that CALLENDAR's assumption is probably the correct one, that the hydration compounds are definite, and that the osmotic pressure of concentrated solutions depends partly upon the hydration value of the molecular complex formed.

If this opinion is correct, then DIETERICH's and RACIBORSKI's figures for the osmotic pressure of saturated NaCl solutions, which are based on vapor pressure determinations, are much nearer the truth than BROWN's figures for the same solution. Although the

difference between the two figures is very large, the former being to the latter as 3:1, inspection of the curves in PHILIP'S little book shows that at a concentration of 700 grams of sucrose per liter of solution, the observed pressure would be to the theoretical pressure calculated on the basis of solution volumes as 3:1. Moreover, the figures of RACIBORSKI for saturated LiCl solutions (80.7 per cent at 20° C.) were obtained by the same methods which DIETERICI used for NaCl; his results give to this solution an osmotic pressure of 965.3 atmospheres. This does not seem too high if NaCl has a value of 375 atmospheres; for NaCl at saturation at 18° C. is a 5.42 M solution, and LiCl a 13.3 M solution (SMITH'S *General chemistry*).

These pressures are of interest on account of their magnitude. If this measure is correct for the osmotic pressure of saturated lithium chloride, then the internal forces causing entrance of water into air-dry *Xanthium* seeds must be at the initial moment in the neighborhood of 965 atmospheres. It is as difficult as it is interesting to think of a saturated aqueous salt solution being as "dry" as an air-dry seed.

From saturated solutions of NaCl these air-dry seeds will imbibe 7 per cent of their air-dry weight. It is readily seen, then, if the pressures given for the two solutions are correct, that the imbibition force in a seed which contains 8-9 per cent of hygroscopic moisture (air-dry) is 965 atmospheres, and that the addition of 7 per cent more water reduces the capillarity and imbibition force from 965 atmospheres to 375 atmospheres, a loss of 590 atmospheres.

The rapid increase of the internal forces with the decreasing water content of the seeds is shown graphically in fig. 8, which is

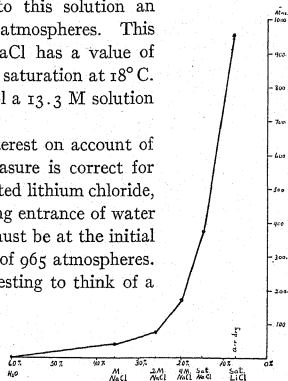


FIG. 8.—Curve of imbibition in M-NaOH, showing the development of osmotically active substances within the semipermeable coat during the second day; the pressure thus developed bursts the outer layers, but leaves the inner one intact.

based upon the figures presented in this discussion. What the initial capillary or surface force would be if all hygroscopic water could be eliminated without changing the fundamental nature of the embryo must be left purely to conjecture; but the figures would probably exceed those which RODEWALD found for starch. He states that dry starch on swelling develops a pressure of 2523 atmospheres (32, p. 227). At present we have no adequate means of measuring such large forces.

### III. Discussion

The results here recorded have a bearing upon many problems in widely separated fields of research. It will be impossible to consider every phase of the subject, and only the more important matters will be considered here.

The general occurrence of non-living semipermeable membranes in plant structures, especially as seed and fruit coats, is of the greatest interest. A large amount of work has been done upon seeds with coats intact, especially upon seeds showing delayed germination, in attempts to stimulate protoplasm to activity. FISCHER (16) used many different kinds of acids, alkalies, and salts on seeds. LEHMANN (26), BECKER (6), and many others have used Knop's solution, etc., as stimulants for resting seeds. In BECKER's paper especially the seeds used by him, mostly Compositae, show such peculiar irregularities of behavior in germination that one cannot escape the conviction that the coats are responsible for much of it. If semipermeability of protective structures is as common as now appears to be the case, much of the work done with salts and other substances acting through the coats will lose considerably in its significance. The very salts which are assumed to enter the seed may be excluded by the testa of the seed. No safe conclusions can be drawn from studies in which the physical characters of the testa are not definitely known. This discovery only emphasizes what the writer has said elsewhere (34) regarding the necessity of removing seed coats when the properties of embryos are being investigated. The testa has physical and chemical characters which may enable it to modify greatly any factor entering into germination behavior, and the effects of these characters must be known before



any sound conclusions can be drawn. Semipermeability is now shown to be common enough that its existence should be proved or disproved before proceeding to use stimuli acting through membranes such as those referred to here.

With a number of the solutions used in these experiments a very high percentage of increase in weight was noticed, running much higher than the imbibition in pure water. This was noticed with  $\text{HgCl}_2$ ,  $\text{AgNO}_3$ , several alcohols, acetic acid, and especially the alkalis. The intake in these cases cannot be considered as due entirely to imbibition and capillarity. Several things may be responsible for the large increase in weight. In the case of the solutions of the heavy metals their specific gravity has something to do with the great increase; but there is convincing evidence that this is not the only cause.

The seeds become greatly swollen, until in many instances they are perfectly cylindrical and stretched to inordinate size. In these cases a fluid is found between the coat and the rest of the seed. Evidently there is a dissolution of various constituents of the embryo whose decomposition products are osmotically active, which exert great pressure upon the coat from within, and which thus cause a very large intake of water. This is true especially when alkalis are used. Fig. 9 shows the curve of increase in weight of seeds kept in molecular  $\text{NaOH}$ . The earlier part of the curve, up to 24 hours, resembles the imbibition curve for water, only the entrance is a little more rapid. Then suddenly the weight increases under the pressure of dissolved organic substances from within, and by the end of three days the outer layers of the coat have been bursted, leaving the inner layer intact. Through its translucent cells one can see the

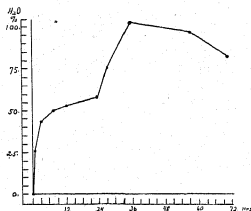


FIG. 9.—Curve showing increase of imbibition force in seeds with decrease of water content; percentage of  $\text{H}_2\text{O}$  is based on absolute dry weight; air-dry seeds are considered to have 8 per cent of hygroscopic moisture in this diagram; the force as here plotted is based on figures discussed in the text.

embryo, not much above normal in size, surrounded on all sides by a liquid which is held in by the semipermeable membrane.

This curve is of great interest in connection with FISCHER's (16) work on the seeds of water plants. When the seeds have a brittle instead of an elastic coat, the pressure from within may be great enough to rupture the coat, and allow oxygen or some other requisite for germination to enter. There is no doubt that this actually happens with acids and alkalies in the case of *Alisma Plantago-aquatica*, one of the seeds which FISCHER stimulated with hydrogen and hydroxyl ions, and which CROCKER (15) showed at the same time needed only to have the coat broken to bring about germination. In view of the effect of certain acids and alkalies on seeds with semipermeable coats, the reason for germination on treatment with these substances is obvious.

In this connection sight must not be lost of the possible effect of acids, alkalies, etc., on the colloids of the seed. FISCHER (17, 18) has shown the remarkable effect of acids and alkalies on the water-holding power of fibrin and frog muscle, and has pointed out the relation of acids to edema and glaucoma (19). A part of the increased weight may be due to the colloids in the embryo holding more water in the different states of ionization induced in them by the presence of acids and alkalies; but most of it is certainly due to the osmotic pressure from within, which adds a large quantity beyond what capillarity and imbibition would take up.

The behavior of the coat in retaining dissolved substances adds further evidence to that already brought forward to show that even after prolonged treatment in tannin solvents the coats are still semipermeable.

The high internal forces in dry seeds is interesting in connection with recent work on the osmotic pressures of the cells of desert plants. FITTING (20) shows that desert vegetation has in many instances cell sap with more than 100 atmospheres of pressure, an adaptation which is of great importance to the plant whose "saturation deficit" must withdraw the water from an arid soil. The value of FITTING's work would have been enhanced if he had used boiling or freezing-point methods as a check to the plasmolytic methods of determining the osmotic pressure. The possibility suggests itself

that the forces which extract water from the soil particles and move it through the plant may not be entirely osmotic whenever the force needed runs high, but may involve capillarity and imbibition as well. A study of surface tension forces in soils should throw some light on this subject.

Many theories have been advanced to explain the cause of semipermeability. Chief among these are (1) the filter or sieve theory, (2) the solubility theory, (3) the "Haftdruck" theory of TRAUBE, and (4) the hydrone theory of ARMSTRONG. Each theory has a certain amount of supporting evidence, and any of them could be applied to the problems of physical or physiological semipermeability.

Since ARMSTRONG (3) used the hydrone theory to explain the semipermeability of the coat of *Hordeum vulgare* as described by BROWN, I have given it more attention than the others, not that it seemed to offer the best explanation, but because of its weakness.

ARMSTRONG's conception of water as a mixture of hydrone, hydrol, and hydronol was developed several years ago (1, 2, 4). The hydrone compounds are composed of  $=OH_2$ , which is related to the hydrones much as  $=CH_2$  is to the carbon compounds. The more complex molecules, as penthydrone, are believed to be inert, resembling the polymethylenes in this respect; whereas the simple hydrones are much more active.

The size of the hydrone molecule is believed to be influenced by temperature. As the temperature of water rises, the complex hydrones are supposed to split up into simpler molecules, which renders the water much more active in all its relations. This conception has received some support recently from the work of BROWN and WORLEY (12), who studied the relation of temperature to the rate of water imbibition by seeds of *Hordeum vulgare*.

Their results indicate that the velocity of water intake may be a logarithmic function of the temperature. The temperature coefficient for the imbibition rate follows approximately the VAN'T HOFF law, indicating that chemical processes are involved in water intake. They suggest that the chemical change thus indicated is the simplification of the complex hydrone compounds into smaller, more active molecules.

Certain solutes are believed to have the same, or similar, effects upon the hydrones as increase in temperature. For instance, acetic acid, and other substances mentioned in this paper as leading to supernormal imbibition, may increase the rate of entrance by breaking down the inert complex molecules of water. If the interpretation of the results of BROWN and WORLEY is correct, the measure of the rate of water intake may be looked upon as a measure of the activity of water when in a certain state which depends on temperature, or on the presence of certain solutes. While this work is of the greatest interest, it is too early to make any general applications of their results.

If the writer understands ARMSTRONG's application of his hydrone theory (3) to semipermeable membranes, the fine particles which make up the membrane are assumed to be chemically united to hydrone or hydrol, perhaps under the influence of surface force. All the intramolecular passageways through the membrane are therefore guarded by hydrone elements. When a salt goes into solution the solute molecules also are believed to be hydrolated in case of non-penetrating solutes. Now if a hydrolated salt presents itself for passage through a hydrolated or hydronated membrane, the salt is seized and held back by the mutual attraction of the hydrolated surfaces of the salt and membrane. If on the other hand an unhydrolated salt presents itself to the same membrane the hydrolated passageways are indifferent to the salt, and it passes through the membrane without the "chemical seizure" retarding its entrance. Selective action would depend upon the salt rather than upon the membrane, for an unhydrolated membrane should allow all salts to pass providing other physical and chemical conditions necessary to passage were met.

The penetrating salts, whether electrolytes or non-electrolytes, are conceived to be those which can exist in water solution in unhydrated condition, or which attract water only to a slight extent; whereas those which are excluded are those which form hydration compounds of considerable stability; and the semipermeability depends on the mutual attraction of hydrated salt and hydrated membrane.

It is the writer's opinion that ARMSTRONG's rather fanciful

theory of the structure of water has not been well received by American chemists; perhaps it has not been given the attention it deserves. As regards its application to the problems of semipermeability there seems to be some difficulty. The data obtained at one point with *Xanthium* seeds argue against the hydrone theory as a universal explanation.

For instance, BROWN found that both NaCl and AgNO<sub>3</sub> failed to penetrate the coat of *Hordeum*. ARMSTRONG says in effect that the membrane is hydronated, both salts are hydronated, therefore they do not enter, although the water in which they are dissolved may enter freely. In my experiments NaCl does not pass through the *Xanthium* coats, therefore both salt and membrane must be hydronated. Now if ARMSTRONG's theory is correct, a solution of AgNO<sub>3</sub> should also be excluded, for BROWN's experiments have shown that AgNO<sub>3</sub> is a hydronated salt when in solution. But this does not happen, for silver nitrate is retarded but slightly, whereas according to ARMSTRONG's hypothesis the mutually hydronated surfaces should attract each other so strongly as to prevent the passage of the salt. Trichloroacetic acid was exceptional in its behavior in BROWN's experiments, so that the hydrone theory cannot as yet be accepted as a general explanation of selective semipermeability. More facts are needed before the physiological semipermeability of living or the physical semipermeability of non-living membranes can be adequately explained.

Further studies on the seed coats of *Xanthium* and other seeds are being continued. The microchemistry of the coat, its relation to oxygen diffusion in respiration, its relation to the impermeability of "dry" solvents, and its use as a measure of surface tension forces in soils are now under investigation. The earlier results of these studies cannot of course be presented at this time.

#### IV. Summary

1. The dry seed coats of *Xanthium* are impermeable to dry alcohol, ether, chloroform, and acetone. BECQUEREL's results with the coats of other seeds are confirmed.
2. Evidence of the diffusion of oxygen through absolutely dry seed coats was not obtained.

3. Selective semipermeability like that found in *Hordeum* has been demonstrated for the seed coat of *Xanthium*.

4. The following substances seem to be excluded: NaCl, CuSO<sub>4</sub>, K<sub>2</sub>CrO<sub>4</sub>, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, glycerol, sugars, HCl, tartaric acid.

5. The following enter, either slowly or rapidly: NH<sub>4</sub>NO<sub>3</sub>, AgNO<sub>3</sub>, NaNO<sub>3</sub>, KNO<sub>3</sub>, KCl, HgCl<sub>2</sub>, FeSO<sub>4</sub>, alcohols, ether, iodine, KOH, NaOH, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, acetic acid, lactic acid, citric acid.

6. The selective activity is independent of any living substance in the seed coat.

7. The coat of *Xanthium* can be removed and used as an osmotic membrane, possessing a great advantage over the coats investigated by BROWN and SCHRÖDER.

8. The testa is composed of three layers, the outer of which cannot function as a semipermeable membrane. The middle layer is several cells thick, the inner layer one cell thick except in the chalazal region. This last layer is probably the nucellar epidermis.

9. By use of strong alkalis the inner membrane can be split loose from the middle layer. Both layers possess osmotic properties, the inner layer in a higher degree than the middle one.

10. Neither layer is as efficient alone as when both are left together. The impairing of the membranes may be due to stretching, or to the effects of the macerating agent.

11. The inner layer is nearly pure cellulose, unsuberized, but perhaps containing some tannin. The middle coat contains more tannin than the inner coat.

12. The tannin does not form a continuous layer in the seed coat. Moreover, treatment with tannin solvents does not destroy semipermeability. The evidence is adverse to REICHARD's view that semipermeability is due to tannin compounds.

13. Semipermeability has been demonstrated for the seed coats of a number of plants in six widely separated families. Many dead plant membranes may possess this property.

14. The capillary and imbibition force of the embryo of *Xanthium* as measured by the osmotic pressure of concentrated salt solutions is about 965 atmospheres when the seed is air-dry.

15. An increase in the moisture of the embryo equal to 7 per cent of its air-dry weight reduces the internal forces by 590 atmospheres.

16. The unusual intake of water noticed with certain substances, especially with certain acids and alkalies, is due largely to the development of osmotically active substances inside the semipermeable membrane.

17. There is some evidence unfavorable to ARMSTRONG'S hydrone theory of selective semipermeability.

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# THE ORIGIN AND DEVELOPMENT OF THE EMBRYO SAC AND EMBRYO OF DENDROPHTHORA OPUNTIOIDES AND *D. GRACILE*. II

HARLAN HARVEY YORK

(WITH PLATE VII)

## Embryo-formation in *D. opuntioides*

Following the arrangement of the nuclei in the micropylar end of the sac, as described above, they enlarge, the one toward the so-called polar nuclei becoming the largest. This nucleus becomes the one functional cell from which the endosperm and embryo are derived. Later the "synergid" and the polar nuclei begin to degenerate and in a short time disappear. Preparatory to division, the functional nucleus becomes very much enlarged and divides by ordinary mitosis, in a plane transverse to the longitudinal axis of the sac (fig. 46). This mitotic division was the first observed following that of the megaspore mother cell. It was not possible to count the chromosomes, but the number was apparently equal to that seen at the division of the megaspore mother cell. The two cells resulting from the division of the functional nucleus divide transversely to the plane of the first division. After a series of divisions a large oval mass of tissue is formed which occupies the upper end of the sac. The cells of this body are large and appear to be exactly alike (fig. 47). After this endosperm-like body has reached about one-twentieth of its size in the mature seed, the cytoplasm of one of the central cells becomes more densely granular than in the others. This cell constitutes the one-celled stage of the embryo (fig. 48). It divides by a wall almost transverse to the longitudinal axis of the flower, forming a 2-celled embryo (fig. 49). A division of these two cells in a plane transverse to the wall which separates them results in the formation of the 4-celled embryo (fig. 50).

The embryo thus arises indirectly from a single cell without fertilization having occurred. The author examined carefully more than 500 plants of *D. opuntioides*, occurring within a radius

of 5 or 6 miles around Cinchona, Jamaica, at altitudes varying from 2000 to 5500 ft. above sea-level, and could find no staminate flowers. Microtome sections of more than 200 ovules mature enough to show whether pollination had occurred gave no traces of pollen adhering to the stigmas or pollen tubes within the tissue of the style. Examination of 150-200 ovules of *D. gracile* likewise failed to show pollen tubes. If pollination does occur in these plants, the mode of origin of the embryo and endosperm is strikingly different from anything elsewhere known. In recent studies on *Phoradendron flavescens*, the author observed near Austin, Texas, a single plant more than a mile distant from others of its kind, fruiting abundantly. The berries of this plant contained normal seeds. Since *Phoradendron flavescens* is dioecious, the chances for wind pollination in this case were very slight. Hence it is apparent that this plant was producing fruit without having been pollinated. In the following year a number of branches bearing pistillate flowers were covered with cheese-cloth bags before the shedding of the pollen had begun, and allowed to remain until there was no longer any possibility of pollination. They produced fruit as abundantly as the uncovered branches. Thus it is evident that in *Phoradendron flavescens* pollination is not necessary for the production of seeds. In fact, pollination probably does not occur in plants under natural conditions, for in studying sections of a few hundred young fruits of this species, no pollen tubes or germinated pollen were seen. There are 18-22 chromosomes present in the cells of the embryo of *D. opuntioides*, which is the number found at the division of the megaspore mother cells. There is thus apparently a constant number of chromosomes throughout all the different stages of development of this plant, which seems clearly to preclude all possibility of fertilization. Since no nuclear fusions were seen within the sac, it seems evident that no reduction division has occurred previous to the formation of the gametophyte. Estimates of chromosomes made in the cells of the embryo of *D. gracile* show that there are 18-20. The cells of the endosperm in each species contain the same number of chromosomes as is found in the embryo.

It was impossible to obtain a complete series of good sections of the different stages in the development of the endosperm and

embryo, owing to shrinkage of the material from lack of penetration of the fixing fluids. The growth of the embryo proceeds very slowly as compared with the endosperm, and it is but a small spherical mass of cells when the latter has come to occupy the whole central region of the carpellary tissue (fig. 51). At first the embryo is intimately connected with the endosperm, from which it gradually separates as development continues. The micropylar end or the radicle is first freed, while the cotyledons are last to be separated. The embryo is strictly dicotyledonous, and when mature is almost entirely imbedded within the endosperm (fig. 52). The endosperm enlarges at the expense of the adjacent tissues. Soon after the origin of the embryo, it begins to elongate in the direction of the longitudinal axis of the ovary and at the same time becomes flattened in the plane of flattening of the spike. Growth at first is almost entirely upward and the apex of the endosperm reaches to the base of the style. Later, by gradually digesting the remains of the placenta, it advances downward into the plate of tracheids at the base of the ovary. The endosperm thus replaces the entire axial region of the flower and lies in direct contact with the vascular traces of the carpels (figs. 20, 21, *tc*) which later form a part of the covering of the seed (figs. 22, 66).

The endosperm is composed of large parenchyma cells, the walls of which are relatively thin and of a most uniform thickness. The cells of the external layer are largest, attaining their greatest size at the base of the endosperm (figs. 53, 54). They are not specially modified for protection as in *Phoradendron flavescens*, and do not form a true epidermis. They fit loosely together and seem to have an absorptive function. This is especially true in the basal portion of the endosperm, in which the outermost layer of cells contains large nuclei and very fine granular cytoplasm which stains brownish yellow in iodine (fig. 53). Since these cells are in direct contact with tracheid cells, the greater bulk of food passing into the endosperm must enter through them. Hence they are considered as being primarily absorptive in function. The fact that the cells of the outer layer fit loosely together also indicates that they are capable of readily absorbing water when germination begins. Sections of seeds which had just begun to germinate showed that

these cells had become turgid and crowded closely together into a very compact layer (fig. 55).

In the ripe seed the outer layer of cells contains large quantities of a fatty substance which stains black in osmic acid and dark blue in cyanin (figs. 53, 54). When germination begins, the fat, as indicated in figs. 53 and 54, almost entirely disappears from them and they become practically colorless. Large brown cystoliths, the chemical nature of which was not determined, were found in some of the cells. The remaining cells contain starch and other food substances which are more or less limited to certain portions. Fatty substances as seen in the outer cells also occur in the inner region of the endosperm and are in greatest abundance in the chalazal part (fig. 53). There is a single layer of cells, densely filled with chloroplasts and starch, subjacent to the outer cells (fig. 55, *cl*). The cells adjacent to the cotyledons (fig. 55, *i*) contain practically no chlorophyll and very little starch, but substances which stain like proteins. Immediately above this layer the cells are abundantly filled with starch (fig. 55, *s*). The remaining portion contains less starch and chlorophyll, but in addition has large cystoliths of calcium oxalate (fig. 55, *cs*). The cells of the embryo are also well supplied with chlorophyll. When germination begins, the chlorophyll present in the seed becomes greatly intensified. It is important to note in this connection that chlorophyll is most abundant in the cells just below the outer layer of cells which become almost colorless when germination occurs. The chlorophyll in the endosperm is manifestly for photosynthetic work, as a result of which food is furnished to the young plant until it becomes fixed on its host. It becomes more evident that the chlorophyll performs the work of photosynthesis from the fact that the seeds will not germinate in the dark nor will germinating seeds continue to develop if kept from the light. A number of attempts to germinate seeds of *D. opuntiioides* in a dark chamber in the laboratory were made, but all failed. Also small dark chambers were constructed out of black paraffined paper over seeds that had been dropped on the branches by birds. At the end of four weeks these seeds had not germinated, while uncovered seeds near those which were covered had germinated. Seeds of *Den-*

*dropemon parvifolius* were placed in the dark chamber with those of *Dendrophthora opuntioides* and germinated as readily as those under normal conditions. Chlorophyll is not present in the endosperm of *Dendropemon parvifolius*, but is abundant in the embryo. From this fact it is seen that the endosperm in *Dendrophthora* and also in *Phoradendron*, which is quite similar, is also a carbon dioxide assimilating organ. It will be of interest to mention here that the radicles of the seedlings of *Dendropemon parvifolius* which germinated in darkness did not turn down toward the substratum, but grew out at various angles. Some, for example, extended upward at an angle of  $90^\circ$ , while others were parallel to the substratum. Thus the bending of the radicles toward the substratum is not due to gravity or to attraction by the substratum, but their behavior seems to be a negative response to the stimulus of light.

DUTROCHET (6) found that the "hypocotyls" of *Viscum album* are negatively heliotropic. His observations were later confirmed by WIESNER (39). The endosperm in its development, as seen in this study, may thus be regarded as being parasitic, drawing its nourishment through its outer layer of haustorial cells from the adjacent tissues. It also becomes a storage tissue, containing an abundance of food material for the embryo and a region in which photosynthesis occurs.

### The viscin

Owing to the lack of fresh material at Baltimore, a detailed study of the viscin has not been possible. Its structure is essentially the same as described and figured by JOHNSON in *Arceuthobium Oxycedri*, and by PEIRCE in *A. occidentale*. Parallel with the maturation of the embryo sac, the cells in the outer half of the carpels become colorless and begin to enlarge (figs. 20, 56). Later they elongate obliquely toward the base of the style when the formation of the embryo begins. In the ripe berry they are greatly elongated and their walls consist of two layers. The outer layer is composed of gelatinous material, while the inner is usually made up chiefly of spiral cellulose thickenings (fig. 57). As a rule the cell cavity is very narrow, and in some cases has been almost obliterated, the cell being chiefly composed of gelatinous material (figs. 58, 59). In some cells the outer wall is spirally thickened.

The inner ends of the cells are attached to a sort of membrane which is two or three cell layers in thickness. PEIRCE has described a similar structure, a "sclerotic membrane," in *Arceuthobium occidentale* as being composed of three or four layers of tannin cells. Apparently there was no tannin in the cells of this body in *Dendrophthora opuntoides*. The viscin in *Phoradendron flavescens* presents essentially the same structure as that here just described, though this was not recognized by the author in his earlier studies on this plant. The layer of viscin surrounding the seed in *Dendrophthora* is much less in proportion to the size of the berry than in *Phoradendron flavescens*. In the latter the viscin evidently prevents extreme drying out and is useful in absorbing water before germination begins. It is not necessary for the germination of the seeds, but if it is stripped from them they will dry up in a few weeks' time when exposed to a dry atmosphere. Seeds from which the pulp has not been removed retain their vitality for an indefinite period of time. In January 1909, the author collected in Austin, Texas, berries of *Phoradendron flavescens* which were dried at room temperature. During June 1910, 12 of the dried berries were soaked in water for 24 hours, after which a part of the pulp was removed and the seeds were placed in a moist chamber. At the end of two weeks 4 of the seeds had begun to germinate. Owing to the warm moist environment of *Dendrophthora opuntoides*, the pulp is probably not an important factor as a means of protection and aid in germination of its seeds, since they germinate almost immediately after being deposited.

The mature berries are bright red in color, oval in shape, 7-9 mm. long, and 6-8 mm. in diameter. Owing to the peculiar order of origin of the flowers, various gradations in the development of fruits, from flowers to ripe berries, often occur in the same spike. The seed is enveloped by a thin layer of viscin, which is surrounded by a thick fleshy pericarp. The latter is covered by a heavily cutinized single-layered epidermis (figs. 22, 66).

#### Embryo-formation in *D. gracile*

The micropylar nuclei of the sac enlarge as much as they do in *D. opuntoides*. Meanwhile the two polar nuclei, which are

located some distance below them, are fusing and migrating upward in the sac (fig. 60). The nucleus resulting from their union lies just below the egg and becomes the nucleus of the primordial cell of the proembryo (figs. 61, 62). Before division it enlarges greatly and is apparently cut off from the sac below by a transverse wall. At the same time two of the micropylar nuclei, the synergids, begin to degenerate. The other nucleus, which is homologous with the one in the sac of *D. opuntioides* from which the proembryo originates, continues to enlarge for a short time as if preparing to divide, and then also begins to degenerate before division of the fusion nucleus occurs. The primordial cell of the proembryo divides by ordinary mitosis (figs. 62, 63). The two resulting cells (fig. 64) by further divisions form a proembryo, which is similar to that of *D. opuntioides* (fig. 65). The development which now follows is also the same as has been given above, hence further descriptions are unnecessary. The origin of the embryo as seen in *D. gracile* suggests the example described by FARMER and DIGBY (8) in *Lastrea pseudo-mas* var. *polydactyla* Wills, in which the embryo arises from a nucleus resulting from the union of two body cells of the prothallus which has come from a normally developed spore and has the haploid number of chromosomes. The difference in the mode of origin of the embryos of *D. gracile* and *D. opuntioides* also recalls that shown by the above mentioned authors between *Lastrea pseudo-mas* var. *polydactyla* Wills and the closely related *Lastrea pseudo-mas* var. *polydactyla* Dadds. In the latter the embryo arises from a projection or budding out of the prothallus which has arisen aposporously. In this same variety these authors also found nuclear fusions occurring in the prothalli which had come from normal spores, quite similar to those in the variety first mentioned. It is probable that a phenomenon similar to that just stated for Dadds' fern occurs in *D. opuntioides*, though no evidence for such a supposition was seen. Owing to the lack of material, it was not possible to make a detailed study of the division of the megaspore mother cell. At the division of the microspore mother cell in *D. gracile* 9 chromosomes were found passing to each pole of the spindle; 18-20 chromosomes were estimated in the cells of the developing embryo. Apparently the same number is also present



in the nuclei of the endosperm. From these facts it is clearly evident that fusion of the polar nuclei in *D. gracile* indicates that the nuclear behavior in the origin of the female gametophyte is the same as in an ordinary sexual plant.

### Discussion

The embryos of *D. opuntioides* and *D. gracile* arise asexually. In the former, reduction division does not occur preceding the formation of the gametophyte, the nuclei of which thus contain the diploid number of chromosomes. The gametophyte is aposporous in origin. Examples of apospory in seed plants have been described by JUEL (16) in *Antennaria alpina*, by MURBECK (22) and STRASBURGER (32) in certain species of *Alchemilla*, by TREUB (34) in *Balanophora elongata*, by OVERTON (27) in *Thalictrum purpurascens*, by LOTSY (19) in *Balanophora globosa*, by OSTENFELD (25, 26), ROSENBERG (29, 30), and MURBECK in species of *Hieracium*, by WINKLER (40, 41) in *Wikstroemia indica*, and by RAUNKIAER (28), MURBECK (23), and JUEL (17) in a number of forms of *Taraxacum*. Apogamy is associated with the aposporous origin of the gametophyte in these forms. In each of these plants, with the exception of *Balanophora*, the embryo arises from what is to external appearances an egg. From a cytological point of view the studies of STRASBURGER and WINKLER are the most important. The former regarded the cell giving rise to the embryo as a sporophytic cell, since it possesses the diploid number of chromosomes. According to this view the embryo may be said to develop by vegetative budding. STRASBURGER regards the process as a case of apogamy and not parthenogenesis as some authors claim. He defines an egg as a cell having the haploid number of chromosomes and capable of being fertilized.

WINKLER disagrees with STRASBURGER in regard to the use of the terms apogamy and parthenogenesis and the theoretical significance of the chromosomes. According to him, apogamy is the origin of a sporophyte from some cell or group of cells of the gametophyte other than the egg. He uses the term parthenogenesis to indicate the formation of an embryo, developed without fertilization, from a cell having the position of an egg, whether the nucleus

of this cell is haploid or diploid. WINKLER also maintains that other factors besides the number of chromosomes determine the character of the gametophyte as well as the sporophyte. YAMANOUCHI'S (42) studies on apogamy in *Nephrodium* support this hypothesis. If we accept STRASBURGER'S narrow definition of the egg, it seems logical to restrict the term gametophyte to structures having the haploid number of chromosomes, that is, to bodies developed after a normal sporogenesis. Regarding *Dendrophthora opuntiioides* from this point of view, a true megaspore formation does not occur, the gametophytic generation itself is omitted, and the nuclei of the embryo sac are thus really vegetative or sporophytic in character. If the egglike cell in *Dendrophthora*, as well as in other similar cases, is a vegetative or sporophytic cell, it is strikingly different in behavior from all other sporophytic cells which we know.

I find no record among seed plants of the origin of an embryo from a cell of an endosperm-like mass, which like that of *Dendrophthora* has been derived from a single egglike cell. If this cell is really a vegetative cell of the sporophyte, we should expect it to develop directly into a sporophyte, as nucellar cells do in *Coelebogyne* and *Funkia*. In *Antennaria alpina* (JUEL 16) or *Thalictrum Fendleri* (DAY 4), for example, the egg cell which develops without fertilization should, if it is really a sporophytic cell, give rise to a sporophyte having the same sex as its parent. Furthermore, the derivation of the endosperm from a single polar nucleus is a peculiar phenomenon if this nucleus be regarded as sporophytic in character.

In the ferns, aposporous prothalli whose nuclei contain the sporophytic number of chromosomes are distinctly different in structure and in possessing sex organs from the parent sporophytes. Hence chromosome number cannot be the sole morphogenic factor in the nucleus. If the nucleus in any way determines structure, it is evident that the nuclei of an aposporous embryo sac must differ in some manner from those of the parent sporophyte, in spite of the identity in chromosome number. Morphologically the nuclei of such an embryo sac may be said to be equivalent in the same sense that the nuclei of a typical embryo sac are. The nucleus from which the embryo sac of *Dendrophthora opuntiioides* develops,

however, is not a mere vegetative one, but is potentially distinct from all other nuclei in the sac. The functional nucleus in the sac of *D. opuntoides* occupies the position of an egg in the usual type of embryo sac. It is different from a diploid egg in that it does not give rise directly to an embryo. It may thus be regarded as a pseudo-egg and the tissue developing from it may be considered a proembryo or pseudo-endosperm. Owing to the very wide range in meaning of the term endosperm, it is perhaps allowable to designate the proembryo as endosperm, although it is not endosperm in the strict sense. TREUB in his studies on *Balanophora elongata* found that one of the polar nuclei gives rise to a mass of tissue which is quite similar to the proembryo described above. He called this body endosperm. The embryo arises from one of the central cells of this body as in *Dendrophthora*. He asserts that the embryo is apogamous in origin. LOTSY arrived at the same conclusions from his studies on *Balanophora globosa*. WINKLER, agreeing with TREUB, designates this mode of origin of the embryo as "somatic apogamy," since the nuclei of the endosperm possess the diploid number of chromosomes. WINKLER applies this same term to the mode of origin of the embryo, for example in that of the *Lastrea pseudo-mas* var. *polydactyla* Dadds, in which the embryo arises by a budding out of the prothallus. Since in *D. opuntoides*, as well as in *Balanophora*, the embryo does not arise directly from the gametophyte, it may be spoken of as pseudo-apogamous in origin.

The fact that there is a fusion of two nuclei in the sac of *D. gracile* preceding the formation of the proembryo is presumptive evidence that warrants the assumption that reduction has taken place in the formation of the megaspores. The nuclei of the sac have thus the haploid number of chromosomes. The nucleus occupying the position of an egg may or may not be capable of being fertilized. The two nuclei which occupy the position of polar nuclei are, as in *D. opuntoides*, derived from the same nucleus. Such a fusion of sister nuclei is a phenomenon of unusual occurrence among plants. It is not unknown among animals. BRAUER (3) has shown that in the majority of parthenogenetic eggs of *Artemia salina* only one polar body is formed; a second one is occasionally

produced. This one, however, never passes out of the egg, but after migrating a short distance its nucleus returns to the egg nucleus and fuses with it, thus restoring the diploid number of chromosomes. BLACKMAN (2) believes that the nuclear fusion occurring in the aecidium of *Phragmidium* is a "reduced form of fertilization." The fusion of two gametophytic nuclei in *D. gracile* is indeed quite comparable to the union of the nucleus of one prothallial cell with that of another in *Lastrea pseudo-mas* var. *polydactyla* Wills, which FARMER and DIGBY designate as pseudo-apogamy. They state:

We would suggest the term pseudo-apogamy to cover instances in which the sexual fusion of gametes is replaced by a fusion of ordinary gametophytic nuclei which morphologically are not sexually differentiated. This would include the process as it occurs in the Uredineae and probably the Ascomycetes, and also the growing number of instances of apogamy in prothallia induced by cultural conditions, as well as such cases of obligate apogamy as that of the *polydactyla* varieties of the male fern.

According to them, conjugation of adjoining cells of the same filament in *Spirogyra* might be looked upon as examples of an analogous phenomenon. Whether the nuclei which fuse in *D. gracile*, in *Lastrea pseudo-mas* var. *polydactyla* Wills, and in *Spirogyra* really are undifferentiated morphologically is not definitely known. The gametes of *Spirogyra* or *Mucor*, for example, are believed to be morphologically alike. Aside from the fact that they are sometimes produced on different individuals, does not their conjugation constitute a process essentially like the examples just mentioned? Physiologically the gametes of *Spirogyra* and *Mucor* are believed to differ distinctly from each other. So in *D. gracile*, the author regards the two gametophytic nuclei which conjugate as potentially different from each other and from all other nuclei in the gametophyte. Externally they are alike. Therefore, may not their fusion constitute a process essentially like that of the conjugation of the gametes in *Spirogyra*? The union of the gametophytic nuclei in *D. gracile* does not result in the mixing of parental characters from separate individuals and does not constitute a true sexual process in this respect. Their fusion results in doubling the number of chromosomes and furnishes a stimulus to further development.

In these respects this process may be said to be like the usual type of fertilization. This conjugation of gametophytic nuclei in *D. gracile* may also in a sense be regarded as a sexual process, in that there is a gametophytic generation followed by a sporophytic in which the sexes are in different individuals.

DEBARY (5) was the first to use the term apogamy, restricting it to cases in which fertilization had disappeared;<sup>1</sup> hence this interpretation does not apply in *D. gracile*. The union of the polar nuclei in this plant should be regarded as a substitution process or pseudo-fertilization, which in some respects is like a true fertilization. The body arising from the fusion nucleus represents a proembryo or pseudo-endosperm, and the embryo is regarded as pseudo-apogamous in origin, as in *D. opuntiioides*. The term pseudo-apogamy is not used here in the sense in which it was used by FARMER and DIGBY. In *Lastrea pseudo-mas* var. *polydactyla* Wills, there is a fusion of nuclei which the author believes is a substituted form of fertilization quite like that in *D. gracile*.

The presence of such a difference in the mode of origin of the proembryo in two closely related species as shown here is indeed a very striking phenomenon; perhaps, however, no more so than the two different modes of origin of the sporophyte in the varieties of *Lastrea pseudo-mas* already mentioned. This phenomenon may be due to the difference in the number of chromosomes present in the gametophytic cells. The fact that in one example development occurs without any sort of fertilization, while in the other it does not take place until the number of chromosomes is doubled, supports the theory that a nucleus must have the sporophytic number of chromosomes for starting the sporophytic generation. YAMANOUCHI's account of the origin of a sporophyte whose cells contain the haploid number of chromosomes from a haploid gametophyte in *Nephrodium* is evidence against this hypothesis. One of the most important problems for solution is the determination of the factors which have led to this difference. Since we know comparatively little of the physiological relation of the Lorantheaceae to their hosts, it is almost useless to attempt an explanation of this phenome-

<sup>1</sup>"Dass einer Species (oder Varietät) die sexuelle Zeugung verloren geht und durch einen andern Reproduktionsprozess ersetzt wird."

non. *D. opuntioides* and *D. gracile* usually occur on different species of plants, hence the food substances which they absorb are probably different in some respects. This difference in the character of food material may have given rise to the difference in the development of these two parasites. In view of the facts just mentioned, such an interpretation seems quite allowable, since the type of reproductive organs formed in *Saprolegnia* has been shown to depend upon the character of the medium in which it is grown.

### Summary and conclusions

The general plane of branching of *Dendrophthora opuntioides* and *D. gracile* is isolateral.

The flower originates a short distance above the axil of the subtending bract.

The floral axis elongates, filling the entire ovarian cavity, and does not become attached to the carpels.

The ovules are greatly reduced, being practically naked nucelli, which are borne on a central placenta. There are two nucelli oppositely placed in the plane of flattening of the "mamelon." In a number of examples vestiges of integuments were found.

A single archesporial cell, hypodermal in origin, arises in each nucellus. The archesporial cell becomes the megaspore mother cell, which gives rise to two megaspores. The one toward the apex of the "mamelon" gives rise to an embryo sac.

Following the formation of the 4-nucleate stage of the embryo sac, the micropylar end of the sac grows almost straight downward until it extends below the level of the insertion of the placenta. It then curves outward into the tissue of the carpel, bends, and grows upward beneath the lining layer of cells of the ovarian cavity until the end of the sac lies almost above the apex of the "mamelon." The embryo sac is hook-shaped; the short arm of which lies within the "mamelon," the long arm in the tissue of the carpel. The short arms of the embryo sac fuse, forming one continuous tube.

There are 7 or 8 nuclei in each sac, 2 antipodals in the chalazal end of the sac, 3 or 4 in the egg apparatus, and 2 polar nuclei.

The 2 antipodal nuclei are sister nuclei and were originally the

2 nuclei present in the chalazal end of the 4-nucleate sac. The 2 polar nuclei are sister nuclei and are derived from one of the 2 nuclei present in the micropylar end of the 4-nucleate sac. The nuclei of the egg apparatus arise from the sister nucleus of the one from which the polar nuclei are derived.

The nuclei of the 2, 4, 7, or 8-nucleate stages of the embryo sac are apparently formed amitotically.

Pollination does not occur.

An embryo is formed in only one of the two sacs in each flower. In *D. opuntioides* the egg nucleus gives rise to a mass of tissue, the proembryo. From a central cell of the proembryo the embryo develops. The endosperm is derived from the remaining cell of the proembryo.

The embryo is regarded as pseudo-apogamous in origin.

In *D. gracile* the "polar nuclei" fuse and give rise to a proembryo quite similar to that in *D. opuntioides*. The embryo and endosperm arise in the same manner as in the latter.

The embryo is strictly dicotyledonous and is almost entirely imbedded in the endosperm.

Apparently there is no chromosome reduction preceding the formation of the megaspores in *D. opuntioides*, while in *D. gracile* this process does seem to occur. In the latter, the diploid number of chromosomes is restored at the fusion of the "polar nuclei."

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## EXPLANATION OF PLATE VII

FIG. 46.—Longitudinal section of two-celled proembryo;  $\times 150$ .

FIG. 47.—Longitudinal section of proembryo;  $\times 150$ .

FIG. 48.—Longitudinal section of proembryo of *D. gracile*, showing one-celled embryo;  $\times 150$ .

FIG. 49.—Longitudinal section of proembryo with two-celled embryo;  $\times 150$ .

FIG. 50.—Longitudinal section of proembryo with four-celled embryo;  $\times 150$ .

FIG. 51.—Outline of longitudinal section of endosperm with inclosed embryo of a young seed, taken from young berry about the size of berry A in fig. 66;  $\times 26$ .

FIG. 52.—Outline of longitudinal section of endosperm with embryo from mature seed, showing distribution of oil globules;  $\times 17.5$ .

FIG. 53.—Portion of longitudinal section of endosperm from mature seed taken at B in fig. 52;  $\times 150$ .

FIG. 54.—Portion of longitudinal section of endosperm from mature seed taken at A in fig. 52;  $\times 150$ .

FIG. 55.—Portion of longitudinal section of germinating seed taken in region C in fig. 52; *cl*, cells abundantly supplied with chloroplasts; *cs*, cells containing cystoliths of calcium oxalate, starch, and small quantities of chloroplast; *z*, cells filled with starch; *e*, cells filled with protein-like substances; *ct*, cells of cotyledons of embryo;  $\times 150$ .

FIG. 56.—Part of longitudinal section of carpel taken at O in fig. 20, showing cells (*vi*) which give rise to viscin;  $\times 150$ .

FIGS. 57–59.—Portions of cells of viscin from ripe seed;  $\times 150$ .

FIG. 60.—Longitudinal section of micropylar end of embryo sac of *D. gracile*, showing fusion of polar nuclei;  $\times 150$ .

FIG. 61.—Longitudinal section of micropylar end of embryo sac of *D. gracile*, showing nucleus (*d*) formed by fusion of polar nuclei; disintegration of egg and synergid just beginning;  $\times 150$ .

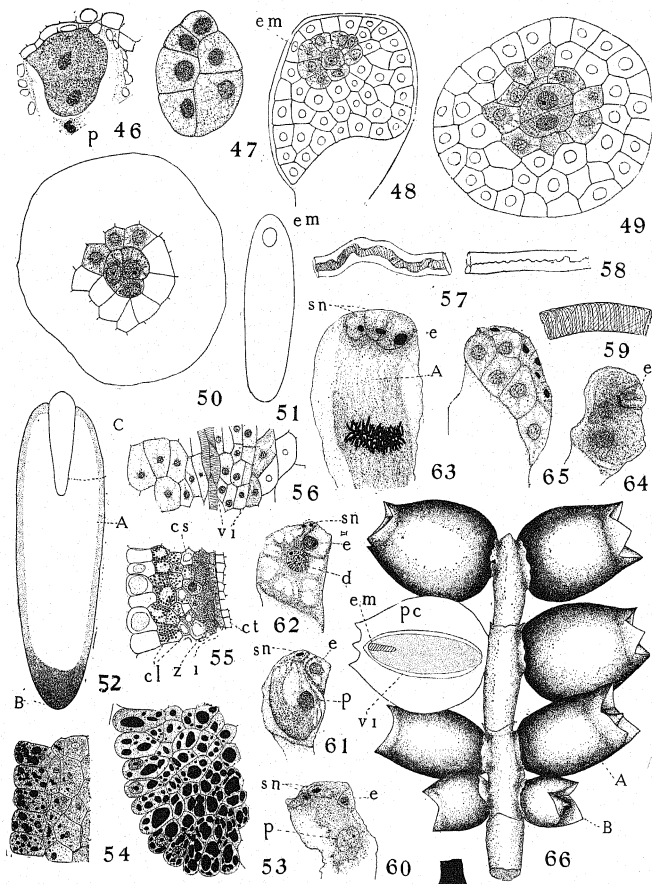
FIG. 62.—Same as fig. 61, with fusion nucleus (*d*) of *D. gracile* preparing to divide; synergids and egg disintegrating;  $\times 150$ .

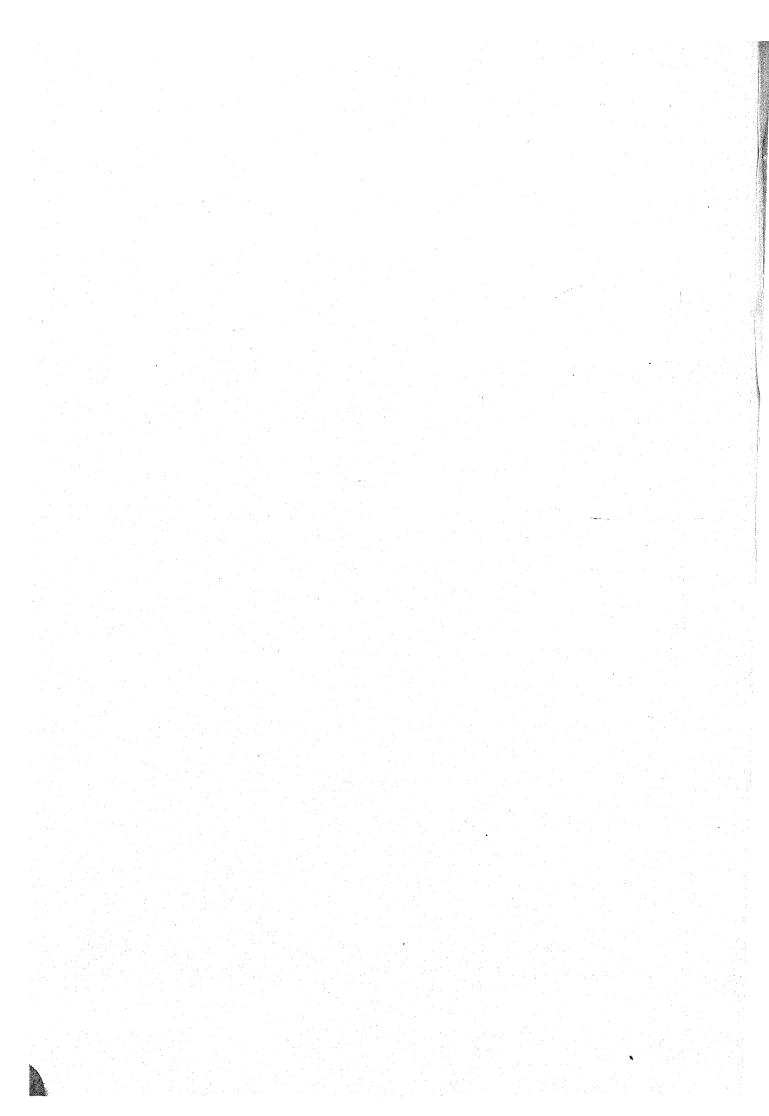
FIG. 63.—Division of fusion nucleus of *D. gracile*: probably displaced downward, from about level A, by fixation; the synergids and egg are disintegrating;  $\times 250$ .

FIG. 64.—Two-celled embryo of *D. gracile*: synergids have disappeared; egg disintegrating;  $\times 150$ .

FIG. 65.—Portion of longitudinal section of proembryo of *D. gracile*, which was composed of about 16 cells;  $\times 150$ .

FIG. 66.—Lateral view of spike with berries in various stages of development; also showing position of endosperm and embryo in relation to the system of arrangement of different members of plant; B, flower in which formation of proembryo has just begun;  $\times 2.8$ .





## XENIA AND THE ENDOSPERM OF ANGIOSPERMS

E. M. EAST

As is well known, the term "xenia" was proposed by FOCKE to describe any effect of pollen of another race upon the tissue of a seed plant apart from that initiating the formation of an embryo. As it has been exceedingly questionable whether any such effect beyond a chemical irritation ever occurs, the word has come to be applied to the appearance of the  $F_1$  hybrid endosperm produced by the fusion of the second male nucleus with the so-called endosperm nucleus of the embryo sac, when its characters are different from those exhibited by the mother plant after self-fertilization.

Since the fact of this fusion was proved cytologically by GUIGNARD (10) and NAWASCHIN (11), data on this type of xenia have interested botanists because of the differences of opinion existing concerning the phylogenetic significance of the angiosperm endosperm.

The most detailed observations on xenia have been those on maize, since numerous maize varieties exist with differences in endosperm characters. The behavior of the following factors in heredity is known from the researches of DEVRIES (5), CORRENS (2), WEBBER (12), EAST and HAYES (7), and EMERSON (9). In addition, EAST (6) has found good indications of at least three additional factors that modify the expression of the red and the purple aleurone colors.

Factor	Action
S.....	Causing full development of starch grains
$Y_1$ .....	Causing yellow color throughout endosperm
$Y_2$ .....	Similar to $Y_1$ but not allelomorphic to it
C.....	Basic color factor necessary for color in aleurone cells
R.....	Present with C gives red color in aleurone cells
P.....	Present with R and C gives purple color in aleurone cells
I.....	Inhibits aleurone color when present with RC or PRC

Observations on crosses wherein these characters have been concerned have made it possible to formulate the following law regarding xenia:

When two races differ in a single visible endosperm character in which dominance is complete, xenia occurs only when the dominant parent is the male; when they differ in a single visible endosperm character in which dominance is incomplete or in two characters both of which are necessary for the development of the visible difference, xenia occurs when either is the male.

It is evident that such a statement can be true only if the two male nuclei always carry the same hereditary factors and if a male nucleus always enters into the formation of the endosperm. The first requirement has been satisfied in every experiment thus far recorded; the second requirement will now be considered.

In particular cases where xenia has followed the crossing of races differing in endosperm color, aleurone color, or ability to mature starch grains, the seeds are not uniform in appearance. One may be half starchy and half wrinkled; another may be half yellow and half colorless; still another may have half of the aleurone cells red or purple and the other half colorless. Examples of this kind are rarely found, although it is a common thing to find seeds with a mottled appearance affecting only the aleurone colors.

CORRENS and WEBBER suggested independently that in these cases the male nucleus may fail to unite with the fusion nucleus and each divide independently, forming either the half-and-half seeds or those which are mottled. WEBBER also suggested, as an alternate hypothesis, the fusion of the male nucleus with one of the polar nuclei, the other polar nucleus remaining independent and dividing.

EAST and HAYES have shown that CORRENS and WEBBER were dealing here with two phenomena. The seeds that are mottled become so only from the development or non-development of color in the aleurone cells. They merely exhibit irregularity of Mendelian dominance, since in some crosses practically all seeds heterozygous for one of the factors producing aleurone color are mottled, although homozygotes are fully colored. Furthermore, the mottling does not extend to the color or other character of the deeper endosperm tissue in case the parental varieties had such differences, which necessarily would be the condition if the endosperm had been formed according to either of WEBBER's independent development hypotheses. This criticism has also been made independently by EMERSON (8).

The other cases, where the endosperm is divided more or less equally into two types, remain to be explained. The hypothesis of independent development of the male nucleus seems improbable if one may judge from relevant cytological data on both animals and plants. The second hypothesis is very plausible. As a third possibility, EAST and HAYES have suggested ordinary "endosperm fertilization" with subsequent vegetative segregation similar to that occurring in bud sports. This could be proved, according to them, if among the  $F_1$  seeds of a cross between parents differing in two allelomorphic pairs, individuals should be found in which the parental characters were combined differently. No such cases have been recorded.

The difficulty of deciding between the first and the second hypothesis of WEBBER lies in the fact that individuals of this kind are very rare, and when they have been found the investigator has not been able to say which particular endosperm character was carried by the male cell and which by the female cell. This was because they have occurred in selfed hybrids where both pollen and egg cells were segregating various Mendelian factors. In the experiments now to be described, this difficulty has been overcome.

The red color in the aleurone cells of maize is due to the interaction of two factors that may be represented by the letters  $C$  and  $R$ ; this color may be changed to purple by the presence of a third factor  $P$ . Red is  $RC$  and purple is  $PRC$ , therefore, although it must be understood both that other factors which have never been lost in any variety may enter into the combination, and that other factors which have been lost in certain varieties may affect the development of color.

Six homozygous white varieties may exist with the following zygotic formulae:  $P\overline{P}R\overline{R}c\overline{c}$ ,  $P\overline{P}r\overline{r}c\overline{c}$ ,  $P\overline{P}r\overline{r}C\overline{C}$ ,  $p\overline{p}R\overline{R}c\overline{c}$ ,  $p\overline{p}r\overline{r}C\overline{C}$ , and  $p\overline{p}r\overline{r}c\overline{c}$ . Any cross between these varieties of such a nature that  $R$  and  $C$  or  $P$ ,  $R$ , and  $C$  are brought together results in the red or the purple color respectively.

Among the selfed maize ears that had been produced in the course of the writer's experiments were a number giving red wrinkled and white wrinkled seeds in the ratio of 3:1. These white seeds must have either the formula  $p\overline{p}R\overline{R}c\overline{c}$  or  $p\overline{p}r\overline{r}C\overline{C}$ .

White seeds from three such ears were planted in isolated plots and used as male parents on the flowers of plants arising from *white* seeds found on selfed ears of 13 other families. A number of these families had the proper formulae to produce color, and about 60,000 red or purple seeds were produced. There were all-purple ears and all-red ears in several families. Other combinations gave purple and white seeds or red and white seeds in the ratio of 1:1. How this came about is clear if one assumes either of the formulae given above for the male parent. Suppose the male parent had the formula  $ppRRcc$ : a family with the formula  $pprrCC$  gives all-red ears, while one with the formula  $pprrCc$  gives ears with red and white seeds in the 1:1 ratio; a family with the formula  $PPrrCC$  gives all-purple ears, while one with the formula  $PprCC$  or  $PPrrCc$  gives ears with purple and white seeds in the 1:1 ratio.

Considering first only the all-purple and the all-red ears, one must conclude that the fusion of the "endosperm nucleus" and the second male nucleus always occurs. If it did not occur, white seeds would result, because a factor from each parent is essential for the production of color.

Among these 60,000 seeds, 6 were found that showed the half-and-half condition; that is, color had developed on one side and not on the other. They were typical illustrations of the phenomenon which WEBBER's two hypotheses were devised to explain. They occurred in only 0.01 per cent of the fertilizations, but in spite of their rarity they show that WEBBER's first hypothesis, assuming independent development of the male nucleus, is untenable, since independent development of the paternal and the maternal nuclei could produce no color. No decision can be made between WEBBER's second hypothesis—fusion of the male nucleus with one polar nucleus and independent development of the other—and the hypothesis of vegetative segregation after partial development. The bilateral symmetry of the halves of the seeds with and without color favors WEBBER's idea; at the same time, it must be pointed out that the frequency of the occurrence is not too great to compare favorably with the frequency with which "bud sports" originate. Though it would afford some satisfaction, a precise explanation of these rare aberrations is not a necessary requisite



to several conclusions indicated by the experiments. It is evident that in the varieties of maize used, a paternal and a maternal nucleus carrying the same hereditary factors as are borne by the true gametes—in the case of the 7 factors investigated—always fuse in the formation of the endosperm. For this reason geneticists investigating maize have been correct in treating the endosperm as if it were an embryo. The endosperm characters have behaved exactly like plant characters. Two white varieties of sweet peas may carry factors both of which are necessary for the production of color. When they are crossed, color develops. Color develops in maize in a quite similar manner when the two complementary factors are carried by the "endosperm nucleus" and the second male nucleus. Nevertheless, one should keep in mind that the problem is complicated. COLLINS (1) found a white ear of maize in a yellow variety that behaved as if its seeds were crossed with the yellow. He interpreted the phenomenon as a mutation showing reversal of dominance, although the data on succeeding generations corroborated those obtained by previous investigators in which yellow was partially or completely dominant. It is not unlikely, however, that COLLINS merely happened upon a plant from white seed in which the male nucleus did not enter into the formation of the endosperm, although other interpretations are possible. This may seem like an odd statement after having shown that the two nuclei always fuse, but it is made advisedly. In most varieties of maize the two nuclei do appear always to fuse, but HAYES is now working out the details in a cross in which a Mexican starchy corn is one of the parents where the nuclei appear never to fuse. In other words, it seems that there may be varieties of maize in which endosperm formation is the opposite of that just described, and within each category *no change to the other has been found*. But may not such a change occur?

Whether or not the last suggestion ever proves to be true, it seems to me that from the data now collected one is entitled to discuss angiosperm endosperm formation from the viewpoint of experimental genetics.

The endosperm of the gymnosperms is essentially vegetative tissue of the female gametophyte. It results from continuous cell

formation originating with the germination of the megaspore, although fertilization occurs during the process. From the time of HOFMEISTER the morphological character of the endosperm of angiosperms was considered to be the same as that of the gymnosperms until the double fertilization was discovered. This fact gave rise to the idea that the angiosperm endosperm might be a sporophytic rather than a gametophytic structure, its nature being that of a monstrous embryo, or possibly that it is a composite tissue neither gametophytic nor sporophytic.

Most botanists, however, have held with STRASBURGER to the original idea that the endosperm is gametophytic. STRASBURGER concluded that the second fusion is not a true act of fertilization uniting the parental qualities and forming an embryo, but a vegetative fusion acting merely as a stimulus to growth. MISS SARGENT, however, believes that it is a degenerate embryo, the monstrous character being caused by the interference of the antipodal nucleus having a vegetative character and an indefinite and usually redundant number of chromosomes in the act.

The difficulty in the situation appears to be the obscurity of the phylogenetic history of the fusion of the two nuclei in the embryo sac and the subsequent fusion with the second male nucleus. The problem is further complicated by the irregularity of endosperm formation in various species. Although triple fusion appears to occur in the majority of angiosperms, the following important general variations have been noted. In addition to these general variations many minor deviations have been found (COULTER and CHAMBERLAIN 4). (1) Vegetative endosperm formation may take place in a similar manner to that occurring in gymnosperms. This may occur without fertilization, or before or after fertilization. Usually the endosperm tissue is formed from the descendants of the antipodal cells, but the chalazal nucleus may degenerate and the endosperm be formed from the micropylar polar nucleus. (2) The polar nuclei may not fuse, but divide independently. (3) Fusion may include many cells.

Furthermore, endosperm formation may be initiated by free nuclear division, or the sac may be divided into two parts by a cell wall after the first division. Even when the latter phenomenon

occurs, endosperm tissue may be formed in both chambers, although usually division proceeds only in the micropylar chamber.

These general cytological data being given, how do the facts from pedigree cultures bear upon the problem?

Just how much weight should be given to data from only one species when discussing the morphological significance of the endosperm is questionable. But in maize it is evident that STRASBURGER's distinction between vegetative and generative fertilization will not hold. Cytological work on other species does not bear out Miss SARGENT's conception, since endosperms form quite regularly without the interference of the antipodal vegetative (?) nucleus. If the perfectly regular manner in which the above-mentioned endosperm characters of maize are transmitted is considered apart from other facts, there appears to be no escape from the conclusion that the endosperm is sporophytic in character. But there is another way of looking at the matter that makes the view of COULTER seem more probable.

COULTER (3) has concluded that conditions in the embryo sac favor fusions of any number or kind of free nuclei—an indefinite process without a necessary phylogeny that results in a growth which is practically gametophytic. It is not dependent upon a male nucleus, a polar nucleus, or even a reduction division.

The experimental evidence accords perfectly with this view. The superficial endosperm characters are indeed transmitted regularly when a male nucleus takes part in the fusion, but there is no reason for believing that the remaining maternal nuclei carry *all* the characters borne by the egg because *these* characters are the same in the nuclei concerned. The egg must usually have an organization somewhat different from that of the other maternal nuclei; although it is recognized that other nuclei sometimes function as eggs. It is likely that a differentiation has ensued which makes a particular nucleus an egg, and that it is not wholly a matter of position. The general belief in the vegetative character of the antipodal cells of the embryo sac is an admission that they have not received *all* the properties retained by other four cells. It is not very heretical, therefore, to assume that the cell that becomes the egg is different from its associates. Botanists hesitate to assume

the differentiation during ontogeny admitted by zoologists. They desire to believe that most plant cells can reproduce the whole plant. But this is a belief and not a fact, and until it becomes a fact it is well to recognize this plausible alternative in considering matters such as periclinal and sectorial chimeras as well as endosperms.

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## BRIEFER ARTICLES

### THE USE OF CELLOIDIN MEMBRANES FOR THE DEMONSTRATION OF OSMOSIS

(WITH THREE FIGURES)

Although the value of a celloidin film as an osmotic membrane is well known to botanists, little use of it has been made on account of the difficulties experienced in manipulation. A description of the method heretofore employed can be found in most textbooks on bacteriology. In this method, a celloidin solution is poured into a test tube or an Erlenmeyer flask and carefully rotated until a film which can be lifted out is formed on the inside of the tube, the sac thus formed being used as the membrane. Another method of preparing celloidin membranes, first described by BIGELOW and GEMBERLING,<sup>1</sup> is generally unknown to botanists, although considerable use has been made of it by chemists. This method consists in pouring a celloidin solution on a clean mercury surface from which the membrane thus formed is removed after the celloidin has sufficiently hardened. I have found that a three-inch Petri dish forms a very convenient container for the mercury. After carefully cleaning the surface of the mercury, enough 10 per cent celloidin solution is poured on it to cover an area 3-4 cm. in diameter. In 2-5 min. the ether and alcohol have evaporated sufficiently to allow the membrane to be lifted. It is important that the celloidin film be "ripe" before attempting to lift it; otherwise a stringy mass will be formed instead of a sheet. As soon as its edge appears scalloped and the surface shows small polygonal patches, the film can be raised. Its general appearance at the time it is "ripe" enough to be taken off is shown in fig. 1.

The best support for the osmotic membrane prepared as described above is the bulb of a thistle tube having a stem a half-inch in length, a thistle tube with a shortened stem being much more easily filled. It can be held in a test tube rack or in a small-necked bottle with the opening of the bulb uppermost and the celloidin membrane placed over its open end (fig. 2, A). The overlapping portion of the membrane should then be pressed to the sides of the flange of the bulb and tied securely with

<sup>1</sup> BIGELOW, S. L., and GEMBERLING, A., Collodion membranes. Jour. Am. Chem. Soc. 29:1576-1599. 1907.

stout Manila twine. As the membrane dries it adheres tightly to the flange, and since it contracts somewhat on drying, it becomes stretched

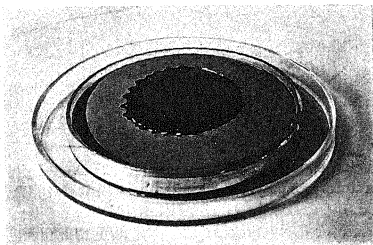


FIG. 1.—Celloidin film on a dish of mercury; the edges show the characteristic appearance at time when it is "ripe."



FIG. 2.—Different stages in the formation of celloidin membranes: *A*, membrane after being placed over the bulb of a thistle tube; *B*, showing how the film adheres to the flange; *C*, after tying the membrane on the thistle tube; *D*, a double membrane.

as taut as the head of a drum (fig. 2, *B*). The ends of the twine are then cut off and the part of the film which extends more than a quarter of an

inch beyond the twine should be trimmed off with a sharp knife (fig. 2, C). This trimmed membrane after further drying for an hour should then be kept in a dish of water until needed for use. MATHEWS<sup>2</sup> dried his membranes over a steam coil for several days, but found that perfectly dry membranes did not allow the solute to pass through very rapidly.

As considerable variation was found in membranes made in the same manner and at the same time, various attempts were made to reinforce them and make their tensile strength more uniform. The method used for testing a membrane consisted in suspending the osmometer and then pouring mercury into the tube until the membrane broke, noting the height of the column of mercury in the tube at that time. Usually there was not a rupture of the entire membrane, but a small leak in one place, and that generally near the flange of the bulb. The different methods used in making the membranes, and their tensile strength, as indicated by the height of the mercury column, are shown in the table given below.

TABLE SHOWING THE RELATIVE STRENGTH OF VARIOUS MEMBRANES

The figures refer to the height (in cm.) of the column of mercury when the membrane broke.

Method of formation	1st	2d	3d	4th	5th	Average
Not tied; put in water 5 min. after being made.....	13.5	8.0	14.0	.....	.....	11.83
Not tied; air dried one hour, then put in water.....	10.5	4.5	11.5	16.0	.....	10.39
Membrane reinforced with bar netting; not tied.....	17.0	12.5	19.0	.....	.....	16.17
Not tied; hardened in chloroform 48 hours.....	15.0	12.5	15.0	.....	.....	14.17
Double membrane; not tied.....	73.0	29.0	73.5	54.0	64.0	58.70
Single membrane, tied; air dried 24 hours.....	9.0	59.0	11.0	8.5	41.0	25.70
Same, then soaked 24 hours in water.....	27.0	8.0	62.0	52.0	22.0	34.20
Double membrane, tied and dried in air an hour.....	94.0	36.0	238.0	230.0*	215.0*	163.40

\* No break in the membrane occurred when the height of the mercury column had reached this point.

As the above tests show, the double membranes are by far the strongest. These are produced by making a second single membrane and superimposing it over one made in the manner described above (fig. 2, D). There may be one or two bubbles of air between the two

<sup>2</sup> MATHEWS, J. H., Osmotic experiments with collodion membranes. Jour. Phys. Chem. 29:281-291. 1910.

membranes, but these do not seem to interfere with the work or the strength of the membrane.

In three days an osmometer made in this manner and filled with a 25 per cent cane sugar solution, under ordinary laboratory conditions, will send up a column of liquid 130-197 cm. high. When similar osmometers are filled with a 10 per cent sodium chloride solution, the liquid will rise 138-172 cm. in the same length of time and under the same conditions. At the end of this time the water column begins to sink slowly.

Attempts were made to render membranes semipermeable by using tannic acid and copper ferrocyanide. Osmometers having membranes prepared as described above were filled with a 5 per cent tannic acid solution and then set in dishes containing the same solution. Enough melted 10 per cent gelatin solution to form a film was poured in the bottom of other osmometers, allowed to harden, and then treated with the 5 per cent tannic acid as before. After standing in the tannic acid solution for 48 hours, the osmometers were placed in water to soak out the excess of tannic acid. The acid leached out for a long time afterward. In some cases the leaching continued for as long as three weeks, even after several changes of water. On account of the difficulty of freeing the membranes of the excess of tannic acid, no further experiments were made with this substance.

For the formation of the copper ferrocyanide membranes, M/20 solutions of copper sulphate and potassium nitrate were used. Osmometers were filled with the copper sulphate solution and immersed in the potassium ferrocyanide solution, while others containing potassium ferrocyanide were placed in the copper sulphate solution (fig. 3). The latter method proved the better, as PFEFFER<sup>3</sup> found in his experiments with porous clay cups. By the end of the third day the celloidin film becomes impregnated with the copper ferrocyanide. There may be some exosmosis of the potassium ferrocyanide and a consequent formation of copper ferrocyanide on the outside of the membrane, but this is easily washed off with water. The precipitation membrane is not formed uniformly throughout the celloidin, but has a mottled appearance at first. Later the membrane becomes uniform in color and texture.

These membranes need not be used at once, but should be used within a month. It was found by BIGELOW and GEMBERLING that for celloidin membranes standing in water for three months the permeability is considerably decreased. The rapidity of the passage of the liquid

<sup>3</sup> PFEFFER, W., *Osmotische Untersuchungen*. Leipzig. 1877.



through the membranes and its consequent rise in the osmometer varies considerably, the osmometers with the smaller, dryer membranes showing a much slower rise. Membranes with a large osmotic surface, for example 5 cm. in diameter, will allow the liquid to rise quite rapidly.

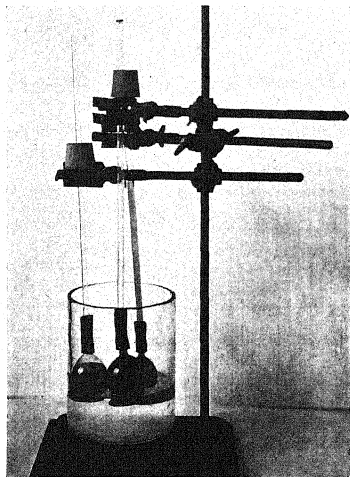


FIG. 3.—Osmometers filled with potassium ferrocyanide solution and immersed in a copper sulphate solution; the precipitation membrane is being formed inside of the celloidin film.

When an osmometer prepared in this manner is set up, water will rise to a much greater height than in those having untreated celloidin membranes. In one experiment with this type of membrane the water rose 8 meters.

I am indebted to Professor J. B. OVERTON for advice and criticism during the progress of this work, for which I take this opportunity of expressing my appreciation.—GILBERT MORGAN SMITH, *University of Wisconsin, Madison, Wisconsin.*

# CURRENT LITERATURE

## BOOK REVIEWS

### Rhythm, periodicity, and zonation

KÜSTER's booklet<sup>1</sup> on zone formation in colloidal media contributes little if anything to botany as such, but is well worth while in presenting a point of view held by a number of continental workers. The first chapter (pp. 23) deals with equidistant zones, the second (pp. 41) with breaks, dislocations, etc., in the zones, the third (pp. 32) with excentric ring systems and polycentric diffusion fields, and the fourth (pp. 7) with zoological considerations and discussion. His point of departure is the Liesegang rings. The concentric rings are produced in a few hours after placing a droplet of 80 per cent  $\text{AgNO}_3$  on 5-10 per cent gelatin plate bearing 0.1 per cent  $\text{K}_2\text{Cr}_2\text{O}_7$ . The rings consist of  $\text{Ag}_2\text{CrO}_4$  and become more definite and more distant from each other as the distance from the drop increases. The author mentions OSTWALD's explanation of the Liesegang rings on the basis of zones of stable, metastable, and labile concentrations; also the fact that the explanation has been questioned.

The bands formed in capillary tubes show definite rhythm and polarity. Aside from the  $\text{Ag}_2\text{CrO}_4$  bands, there are others caused by impurities in the gelatin which KÜSTER terms small rhythms, in contrast to the great rhythms of  $\text{Ag}_2\text{CrO}_4$ . Many conditions modify the patterns given by the precipitate of  $\text{Ag}_2\text{CrO}_4$ : contact with the dish in contrast to free gelatin, presence of foreign bodies in the gelatin, tensions and pressures, and others. In fact, patterns can be produced that resemble closely all the various patterns appearing in mottled leaves, in the arrangement of vascular elements of plants, and in markings of tracheae.

The writer emphasizes the fact that the pattern differentiation of  $\text{Ag}_2\text{CrO}_4$  precipitate shown in the gelatin plate is a self-differentiation occurring under constant environmental conditions. This is taken to show that similar periodic or rhythmic structural changes in the cell may be independent of rhythm in the environment, a matter of self-differentiation. The work also indicates the possibility of the independence of such differentiation from vitalistic peculiarities. The fact that a simple diffusion process in gelatin gives complex polarized precipitation patterns leads the author to conclude that perhaps the complex patterns in the organism may likewise be referred to a simple diffusion

<sup>1</sup> KÜSTER, ERNST, *Über Zonenbildung in kolloidalen Medien. Beiträge zur entwicklungsmechanischen Anatomie des Pflanzen* (erstes Heftes). pp. x+111. figs. 53. Jena: Gustav Fischer. 1913.

process in a colloidal matrix, rather than explained by appeal to complex regulation processes. While this work deals in the main with analogies in morphological features, the writer emphasizes the fact that dynamic activities in the non-living often show rhythmic action determined by internal characters of the system rather than change of the environment. He cites, along with several other examples, the periodic elimination of oxygen when a clean mercury surface is covered with a neutral or slightly alkaline solution of hydrogen peroxide. In his later work the writer will undoubtedly turn more to the dynamic.

Rhythm in plant activity is held by many to be generally related to environmental rhythm. Others feel that rhythm is a necessity of the very nature of protoplasmic activity; activity must be followed by rest. An examination of the facts that KÜSTER offers shows the possibility of rhythms in the organism independent of environmental rhythm, and as well independent of the inscrutable features of protoplasm. In short, internally determined rhythms may be matters of relatively simple chemical and physical laws.—WILLIAM CROCKER.

#### Makers of British botany

Under this title, Professor OLIVER has edited a most interesting book.<sup>2</sup> The book grew out of a series of ten lectures delivered by various botanists at the University College of London in 1911. These lectures have been supplemented by six other chapters, so that the work might be more fully representative.

The botanists included and those who prepared the sketches are as follows: ROBERT MORISON (1620-1683) and JOHN RAY (1627-1705), by S. H. VINES; NEHEMIAH GREW (1641-1712), by Mrs. ARBER; STEPHEN HALES (1677-1761), by FRANCIS DARWIN; JOHN HILL (1716-1775), by T. G. HILL; ROBERT BROWN (1773-1858), by J. B. FARMER; Sir WILLIAM HOOKER (1785-1865), and Sir J. D. HOOKER (1817-1911), by F. O. BOWER; J. S. HENSLOW (1796-1861), by GEORGE HENSLOW; JOHN LINDLEY (1799-1865), by FREDERICK KEEBLE; WILLIAM GRIFFITH (1810-1845), by W. H. LANG; ARTHUR HENFREY (1819-1859), by F. W. OLIVER; WILLIAM HENRY HARVEY (1811-1866), by W. LLOYD PRAEGER; MILES BERKELY (1803-1889), by GEORGE MASSEE; Sir JOSEPH GILBERT (1817-1901), by W. B. BOTTOMLEY; W. C. WILLIAMSON (1816-1895), by D. H. SCOTT; HARRY MARSHALL WARD (1854-1905), by W. THISELTON-DYER; The Edinburgh professors (1670-1887), by I. BAYLEY BALFOUR.

It is impossible to review such a book in a brief space, but botanists will be glad to know where biographies of these botanists may be obtained. Each one stood for some phase of botany and contributed his share to its history.—J. M. C.

<sup>2</sup> OLIVER, F. W., *Makers of British botany*, a collection of biographies by living botanists. 8vo. pp. 332. *pls.* 26. Cambridge: University Press. 1912. 9s.

### Protein metabolism

CATHCART<sup>3</sup> has added a volume on metabolism of proteins to the series of monographs on biochemistry being issued by the publishers cited. There are nine chapters under the following headings: digestion and absorption of proteins, protein regeneration, feeding experiments with abietur products of digestion, deamination, influence of food on the composition of the tissues, protein requirements, theories of protein metabolism, starvation, work.

The author says: "The present monograph does not pretend to cover the whole literature of protein metabolism; it consists rather of the discussion of the more important results published during the last decade." Yet the bibliography includes 430 citations. This indicates how extensive is the work done on the protein metabolism of mammals alone as compared with the scattering contributions to protein metabolism in plants. This little volume makes readily available to plant physiologists the status of our knowledge of this subject in mammals. It brings one to the realization of some of the striking similarities between protein metabolism in plants and in mammals, as for instance the power of deamination. One also comes to appreciate some of the differences appearing in the two groups of organisms, as is shown in the power of synthesizing amino acids.—WILLIAM CROCKER.

### Herbals

Mrs. ARBER<sup>4</sup> has brought to the knowledge of botanists some very interesting information concerning the early history of botany. She has given a general sketch of the evolution of the printed herbal from 1470 to 1670 from a botanical and also an artistic standpoint. Many of these herbals are extremely rare, and Mrs. ARBER has done good service in making them more real to botanists. Of course the text is full of interest, but the numerous illustrations introduce one even more vividly into the botanical atmosphere of three or four centuries ago.

A list of the nine chapter titles will give a better conception of the contents of the volume: (i) "The early history of botany"; (ii) "The earliest printed herbals"; (iii) "The early history of the herbal in England"; (iv) "The botanical renaissance of the sixteenth and seventeenth centuries"; (v) "The evolution of the art of plant description"; (vi) "The evolution of plant classification"; (vii) "The evolution of the art of botanical illustration"; (viii) "The doctrine of signatures, and astrological botany"; (ix) "Conclusions." —J. M. C.

<sup>3</sup> CATHCART, E. P., *The physiology of protein metabolism*. pp. viii+142. New York: Longmans, Green & Co. 1912.

<sup>4</sup> ARBER, AGNES, *Herbals, their origin and evolution, a chapter in the history of botany (1470-1670)*. 8vo. xviii+253. pls. 22. figs. 113. Cambridge: University Press. 1912.

## MINOR NOTICES

**The fresh-water flora of Germany, Austria, and Switzerland.**—PASCHER<sup>5</sup> has begun the publication of a series of brochures dealing with the fresh-water flora of Germany, Austria, and Switzerland, assisted by numerous specialists. The plan includes 16 parts, 4 of which have just appeared: no. 2 (pp. 192. figs. 398), Flagellatae 2, by A. PASCHER (Chrysomonadinae, Cryptomonadinae, and Chloromonadinae) and E. LEMMERMANN (Eugleninae); no. 3 (pp. 66. figs. 69. M 1. 80), Dinoflagellatae (Flagellatae 3), by A. J. SCHILLING; no. 9 (pp. 51. figs. 89. M 1. 80), Zygnemales, by O. BORGE and A. PASCHER; no. 10 (pp. 192. figs. 398. M 4), Bacillariales (Diatomeae), by H. v. SCHÖNFELDT. The numerous illustrations and analytical keys should make the recognition of forms comparatively easy.—J. M. C.

**William Russell Dudley.**—Leland Stanford Junior University has published a "Dudley Memorial Volume," containing a paper by the late Professor DUDLEY and appreciations and contributions by friends and colleagues. The list of scientific papers is as follows: "The vitality of *Sequoia gigantea*," by W. R. DUDLEY; "The morphology and systematic position of *Calycularia radiculosa*," by D. H. CAMPBELL; "Studies of irritability in plants. III. The formative influence of light," by G. J. PERCE; "The gymnosperms growing on the grounds of Stanford University," by LEROY ABRAMS; "The Synchronia in the vicinity of Stanford University," by JAMES McMURPHY; "The law of geminate species," by D. S. JORDAN; "Some relations between salt plants and salt spots," by W. A. CANNON; "North American species of the genus *Amygdalus*," by W. F. WIGHT.—J. M. C.

## NOTES FOR STUDENTS

**Cultures of the Uredineae.**—The publications of 1912 on cultural work with the plant rusts show an increasing tendency in all parts of the world to clear up by systematic efforts rather than by sporadic cultures the problems of biological relationships in this group of parasitic fungi. In the United States, ARTHUR, who for many years has been prominently associated with this field of research, reports<sup>6</sup> six species which either have been shown to be autoecious or have been connected with their antithetic generation for the first time. These are as follows: *Puccinia Lygodesmiae* Ellis et Ev. from *Lygodesmia juncea* (Pursh) D. Don produced teleutospores on the same host without the intercalation of pycnidia or other spore forms. *Aecidiospores* of *Aecidium monoicum* Peck from *Arabis* sp. produced uredinia and telia on

<sup>5</sup> PASCHER, A., Die Süßwasser-Flora, Deutschlands, Oesterreichs, und der Schweiz. Parts 2, 3, 9, and 10. Jena: Gustav Fischer. 1913.

<sup>6</sup> ARTHUR, J. C., Cultures of Uredineae in 1911. *Mycologia* 4:49-65. 1912.

<sup>7</sup> Unless otherwise stated, teleutosporic material was used in making the infections and the teleutosporic host is given first.

*Trisetum subspicatum* (L.) Beauv. and *T. majus* (Vasey) Rydb.; the species is described as *Puccinia monoica* (Peck) Arthur. *Gymnosporangium Nelsoni* Arth. (*G. durum* Kern) from *Juniperus uahensis* (Engelm.) Lemmon produced only pycnidia on *Amelanchier vulgaris* Moench. *Gymnosporangium effusum* Kern from *Juniperus virginiana* L. produced only pycnidia (*Roestelia transformans* Ellis?) on *Aronia arbutifolia* (L.) Ell. *Gymnosporangium gracile* (Peck) Kern et Bethol. from *Juniperus monosperma* (Engelm.) Sarg. produced pycnidia and aecidia on *Philadelphus coronarius* L. In addition to these, cultures of 15 species are reported as confirming previous work.

FRASER,<sup>8</sup> who has been investigating the rusts in Canada, especially those inhabiting conifers, reports the following species whose relations have been worked out for the first time. *Necium Farlowii* Arthur from *Tsuga canadensis* sown on the same host produced only teleutospores but no pycnidia. *Melampsoropsis* (*Chrysomyxa*) *Pyrolae* (DC.) Arth. from *Pyrola americana* Sweet and *P. elliptica* Nutt. produced pycnidia and aecidia (*Peridermium conorum-Piceae* [Schw.] Arth.) on cones of *Picea mariana* (Mill.) B.S.P. and *P. canadensis* (Mill.) B.S.P. *Pucciniastrum minimum* (Schw.) Arth. from *Rhodora canadense* (L.) B.S.P. produced aecidia (*Peridermium Peckii* Thüm.) on leaves and cones of *Tsuga canadensis* (L.) Carr. *Uromyces Spartinae* Farl. from *Spartina Michauxiana* Hitch. infected *Arenaria lateriflora* L., but failed to infect *Spergularia canadensis* (Pers.) Don, while the same rust from *Spartina patens* (Ait.) Muhl. and *S. glabra* var. *alterniflora* (Loisel) Merr. infected *Spergularia canadensis*, but failed to infect *Arenaria lateriflora* L. *Melampsora arctica* Rostr. from *Salix discolor* Muhl. produced pycnidia and aecidia (*Caeoma* sp.) on *Abies balsamea* (L.) Mill. *Melampsora* (*Medusae* Thüm?) from *Populus grandidentata* Michx. produced pycnidia and aecidia (*Caeoma Abietis canadensis* Farl.) on *Tsuga canadensis* (L.) Carr., and in one case pycnidia on *Larix laricina*. Cultures confirming former work are reported for 12 species.

Of great interest is the discovery of the relation between certain fern rusts and the aecidial forms known as *Peridermium balsameum* Peck on *Abies balsamea* (L.) Mill. This relationship, suggested in this paper, is confirmed in a later note,<sup>9</sup> in which it is stated that *Uredinopsis Osmundae* Magnus, *U. Struthiopteridis* Störmer, *U. Phegopteridis* Arthur, *U. mirabilis* (Peck) Arthur, and *U. Atkinsonii* Magnus have as their aecidial stages forms of *Peridermium balsameum* Peck on *Abies balsamea*. A complete account is promised.

HEDGCOCK<sup>10</sup> succeeded in establishing the relation between *Peridermium filamentosum* Peck and the *Cronartium* on species of *Castilleja*. The aecidi-

<sup>8</sup> FRASER, W. P., Cultures of heteroecious rusts. *Mycologia* 4:175-193. 1912.

<sup>9</sup> ———, Note on the life histories of the fern rusts of the genus *Uredinopsis*. *Science* N.S. 36:595. 1912.

<sup>10</sup> HEDGCOCK, G. C., The *Cronartium* associated with *Peridermium filamentosum* Peck. *Phytopathology* 2:176-177. 1912.

ospores were sown on two unidentified species of *Castilleja* and produced uredinia of a species of *Cronartium* for which the name *C. filamentosum* is proposed.

In Europe several papers on cultural work with rusts have appeared. Prominent among them is a paper by KLEBAHN,<sup>11</sup> who reports the results of cultural experiments carried out from 1907 to 1911. Many of the results are new, while others confirm or supplement the work previously published by the author or other investigators. Unfortunately, the presentation is not sufficiently clear and concise to enable one to distinguish those which are new from those which confirm other work. In this respect the reports of ARTHUR, and likewise that of FRASER, might well serve as models. The main results given in the paper are summarized here. *Uromyces Pisi* (Pers.) DeBary, sown on the young subterranean shoots of *Euphorbia Cyparissias* L. in the spring of 1906, produced the usual deformations with pycnidia and aecidia in the spring of 1907, thus confirming the work of JORDI. Aecidiospores from this culture infected *Pisum sativum* L. In another experiment *Lathyrus vernus* Bernh. was infected by aecidiospores from *E. Cyparissias* collected in another locality. This result suggests that several forms of *Uromyces* have their aecidia on that host. *Uromyces lineolatus* (Desm.) Schroet. from *Scirpus maritimus* L. infected *Berula angustifolia* Koch, *Oenanthe aquatica* Lamarck, and *Hippuris vulgaris* L. Aecidiospores of *Puccinia argentata* (Schultz) Winter from *Adoxa Moschatellina* L. infected leaves of *Impatiens noli-tangere* L. and the subterranean shoots but not the developing leaves of *Adoxa*. It appears, therefore, that the infection of *Adoxa* takes place only in the subterranean parts. A form of *Puccinia* on *Carex crinita* Lam. infected *Ribes aureum* Pursh, *R. alpinum* L., *R. Grossularia* L., and *R. Cynosbati* L. This type, which was described by ARTHUR as *P. albiperidia*, the author does not regard as distinct from *P. Ribesii-Caricis* Kleb. Another form which had not been tested, and which was collected on *Carex teretiuscula* Gooden., produced aecidia on *Ribes rubrum* L., *R. alpinum* L., and also on *Urtica dioica* L. This was therefore a mixture of *P. Ribesii-Caricis* and *P. Urticae-Caricis*. A *Puccinia* from *Carex ligERICA* Gay produced aecidia on *Taraxacum officinale* Web. This rust, therefore, belongs to *Puccinia silvatica* Schröter, which is a collective species whose forms have not been separated. *Puccinia Polygoni amphibii* Pers. on *Polygonum amphibium* L. infected *Geranium Phaeum* L., *G. pratense* L., and *G. sanguineum* L., while *Puccinia Polygoni* Alb. et Schwein. from *Polygonum convolvulus* infected *Geranium molle* L., but none of the others.

The existence of specialized forms of *Puccinia Smilacacearum-Digraphidis* Kleb. was confirmed in the following manner. Aecidiospores from *Paris quadrifolia* L. produced an abundance of teleutospores on *Phalaris arundinacea*. The teleutospores from this culture produced aecidia freely on *Paris quadri-*

<sup>11</sup> KLEBAHN, H., Kulturversuche mit Rostpilzen. Zeitschr. Pflanzenkrankh. 22:321-350. 1912.

*folia* and *Polygonatum multiflorum*, but sparsely on *Convallaria majalis* L. and *Majanthemum bifolium* Schmidt. Teleutospores of the same species from another region infected *Convallaria* freely, but produced only yellow spots on the other three liliaceous plants. Aecidiospores of *Puccinia Symphyti-Bromorum* F. Müller from *Symphytum officinale* L. infected *Bromus inermis* Leyss., *B. erectus* Huds., *B. rigidus* Roth, and *B. mollis* L., thus confirming the relation between this aecidium and the brome rusts. Out of 15 species of plants on which *Puccinia persistans* Plowright from *Agropyrum repens* Beauv. was sown, only *Thalictrum flavum* L. was infected.

With the crown rusts of grasses the following cultures were made. (1) Aecidiospores obtained by sowing *Puccinia coronata* f. *agrostidis* Erikss. from *Agrostis vulgaris* With. on *Rhamnus Frangula* (*Frangula Alnus* Mill.) infected only *Agrostis alba* L. of the 8 grasses used. Since plants of *Calamagrostis* were not infected, this result is further evidence for the distinctness of the forms of crown rusts on *Agrostis* and on *Calamagrostis*. (2) Aecidiospores obtained by sowing *Puccinia coronifera* f. *Lolii* Erikss. on *Rhamnus cathartica* L. infected *Lolium perenne* L., *L. temulentum* L., *Festuca elatior* L., and *Holcus lanatus* L. This confirms the results of other experiments that the crown rusts known as the forms *Lolii* and *Festucae* are not well differentiated. (3) Aecidiospores obtained by sowing *Puccinia coronifera* f. *Holci* Kleb. from *Holcus lanatus* L. on *Rhamnus cathartica* infected both *Holcus lanatus* and *Lolium perenne*, showing that the forms *Holci* and *Lolii* are likewise not sharply differentiated. (4) A crown rust on *Arrhenatherum elatius* Mert. et Koch was found to produce aecidia on *Rhamnus cathartica*, and thereby proved to belong to *Puccinia coronifera* Kleb. Since the aecidiospores infected *Arrhenatherum elatius* but none of the other grasses subject to the attacks of crown rusts, this is considered by the author as a special form, *Arrhenatheri*.

Cultures with *Phragmidium Rubi* (Pers.) Winter, *P. violaceum* (Schultz) Winter, and *Kuehneola albida* (Kühn) Magnus on some 26 species of *Rubus* showed that no specialized races exist within the species in these rusts; the species, however, show differences in their behavior toward different species of *Rubus*. *Phragmidium Rubi* infected *Rubus caesius* L. and the species of the *CORYLIFOLII* group, but only rarely the species of other groups. *P. violaceum* infected most of the other species but not *R. caesius*, and only rarely the members of the *CORYLIFOLII* group. *Kuehneola* infected nearly all the species. Sowing of aecidiospores of *Melampsora vernalis* Mieszl. from *Saxifraga granulata* L. on the same host confirmed the experiments of PLOWRIGHT and of DIETEL according to which this rust is autoecious. A number of cultures did not give positive results. Sowings of uredospores of *Uromyces Alchemillae* (Pers.) Winter on *Alchemilla* gave no infection, although successful infections have been previously reported both by the author and by FISCHER. The life history of this fungus, whose mycelium is perennial in the rootstocks of *Alchemilla*, has not been sufficiently cleared up. *Puccinia Tanacetii* DC. from *Tanacetum*



*vulgare* L. failed to infect that host. *P. Pringsheimiana* Kleb. grown on *Carex acuta* L. failed to infect *Ribes nigrum* L., although *R. Grossularia* L. was freely infected. An attempt to confirm LIRO's observation that *Pedicularis palustris* L. is the teleutospore host of *Peridermium Pini* Willd. (Kleb.) was not successful. Sowings of aecidiospores on a number of other possible hosts resulted in failure, so that the teleutospore host of this important rust is still unknown. Aecidiospores of *Aecidium Circaeae* Ces. from *Circaea lutetiana* L. failed to infect *Brachypodium silvaticum* R. Sch., which was regarded as a possible alternate host for this fungus.

In conclusion, the author adds some observations on the wintering of uredospores of grain and other rusts. The uredospores of *Melampsoridium betulinum* (Pers.) Kleb., *Melampsora Larici Tremulae* Kleb., *Thecopsora Vaccinii* (Alb. et Schw.) Winter, and *Kuehneola albida* (Kühn) Magnus were incapable of surviving the winter. Some observations on an unknown species of *Melampsora* on *Populus alba* L. indicate that the mycelium of this fungus persists in the buds and produces uredinia on the unfolding leaves. All attempts to infect grasses with teleutospores of *Puccinia graminis* failed. To account for the appearance of the grain rusts in spring it has been suggested that the fungus is carried over with the seed. To test this hypothesis further, the author planted a number of plots with seed from plants badly infected with rust, but the young plants were free from rust in every case.

FISCHER publishes two papers on the specialization of certain rusts. The first<sup>12</sup> deals with specialization of forms within the collective species *Puccinia Saxifragae* Schlecht. inhabiting various European species of *Saxifraga*. DIETEL and H. and P. SYDOW have shown that the forms associated under that name can be separated into a number of species morphologically well marked. The possibility that within these species there might exist physiological races not morphologically distinguishable led FISCHER to investigate the *Puccinia* on *Saxifraga stellaris* in Norway. Sowings of the teleutospores were made on *S. stellaris*, *S. rotundifolia*, *S. androsacea*, *S. nivalis*, and *Aizoon longifolia*. Only *S. stellaris* was infected. In the course of these experiments, FISCHER observed that the newly formed teleutospores germinated immediately and continued during the entire summer to infect the leaves of the plants on which they were borne. The teleutospores of this species, therefore, act indifferently like those of a micro-*Puccinia* or of a lepto-*Puccinia*. A differentiation of the teleutospores into persistent and caducous types, which occurs in other species with teleutospores of similar behavior, does not occur in this species.

In the second paper<sup>13</sup> FISCHER gives further results of his investigations on the life histories of forms of *Uromyces caryophyllinus* (Schrank) Winter.

<sup>12</sup> FISCHER, ED., Beiträge zur Biologie der Uredineen. 2. Zur Biologie von *Puccinia Saxifragae* Schlecht. Mycol. Centralbl. 1:277-284. 1912.

<sup>13</sup> ———, Beiträge zur Biologie der Uredineen. 3. Die Specialization des *Uromyces caryophyllinus* (Schrank) Winter. Mycol. Centralbl. 1:307-313. 1912.

Formerly FISCHER<sup>14</sup> had shown that the aecidiospores of the aecidium on *Euphorbia Gerardiana* collected in a district of Switzerland infected *Saponaria ocymoides*, but not other members of the pink family. Later, in a preliminary paper,<sup>15</sup> he described the successful infection of *Tunica prolifera* by aecidiospores from the same *Euphorbia* collected in another region (near Heidelberg). The existence of specialized forms within the species *Uromyces caryophyllinus* suggested by this experiment was confirmed by the experiments reported in the present paper. In this series of cultures the aecidiospores from *Euphorbia Gerardiana* collected near Heidelberg were sown on plants representing 10 species of the Caryophyllaceae, but only *Tunica prolifera* was infected. In single instances one uredo pustule was observed on *Saponaria ocymoides* and one on *Tunica Saxifraga*. A repetition of sowings of aecidiospores, collected in the region from which the material first used to infect *Saponaria ocymoides* was obtained, resulted in a doubtful infection of *Saponaria*, but a fairly abundant infection of *Tunica prolifera*. It is probable, therefore, that this material consisted mostly of the *Tunica*-form. These experiments show that *Uromyces caryophyllinus* consists of at least two biologic forms, one *Tunica prolifera*, rarely infecting *Saponaria ocymoides*, and one on *S. ocymoides*, whose relations to *Tunica prolifera* are not fully known.

SCHNEIDER<sup>16</sup> reports the following results of cultures of rusts infecting liliaceous plants. *Uromyces Scillarum* (Grev.) Winter, which occurs on various species of *Muscari* and *Scilla*, infected only *Muscari racemosum*, the species from which the material had been obtained. The teleutospores were found to germinate either immediately or after a period of dormancy. *Puccinia Schroeteri* Passerini from *Narcissus radiiflorus* infected also *N. pseudonarcissus*. *Puccinia Allii* (DC.) Rudolphi is reported by Sydow as occurring on 27 species of *Allium*. Teleutospores from *Allium sphaerocephalum* produced uredinia on that species and also on *A. sativum*, *A. hymenorrhizum*, *A. oleraceum*, and *A. fistulosum*. In one case pycnidia and aecidia appeared on *A. sativum*. Uredospores of *Puccinia Porri* (Schw.) Winter from *Allium Schoenoprasum* infected *A. Schoenoprasum*, and to a less degree *A. ampeloprasum*, *A. sphaerocephalum*, *A. strictum*, *A. montanum*, *A. fistulosum*, *A. oleraceum*, and *A. hymenorrhizum*. In one case, a sowing of teleutospores from *A. Schoenoprasum* on plants of that species resulted in the production of aecidia. This fact is of interest, since TRANZCHELL,<sup>17</sup> as a result of experiments with the same form, found that no aecidia were formed, whence he concluded that this was a true hemi-*Puccinia*. The existence of hemi-forms is therefore still in doubt.

<sup>14</sup> Rev. Bot. Gaz. 53:79. 1912.

<sup>15</sup> FISCHER, ED., Über die Spezialisierung des *Uromyces caryophyllinus* (Schrank) Winter. Mycol. Centralbl. 1:1, 2. 1912.

<sup>16</sup> SCHNEIDER, W., Zur Biologie der Liliaceen bewohnenden Uredineen. Centralbl. Bakt. 32:452, 453. 1912.

<sup>17</sup> Rev. Bot. Gaz. 53:80. 1912.

TREBOUX,<sup>18</sup> working at Nowotscherkassk in southeastern Russia, reports the results of a number of infection experiments carried out in that region. Since the literature on the subject was not available to him, he was unable to consider the work of former investigators. The following is a list of the successful infections. Aecidiospores from *Ranunculus illyricus* L. infected *Festuca ovina* L. (*Uromyces Festucae* Syd.). Aecidiospores from *Sium lancifolium* MB. infected *Scirpus maritimus* L. (*Uromyces lineolatus* [Desm.] Schroet.). Aecidiospores from *Euphorbia virgata* W.K. infected *Astragalus hypoglottis* L. (*Uromyces Astragali* [Opiz.] Sacc.). In former experiments, uredospores from *Astragalus virgatus* Pall. infected *A. cicer* L., *A. glycyphyllus* Pall., *A. ponticus* Pall., *A. cruciatus* Link., *A. hamaeus* L., *A. placatus* Lam., *A. tianchanicus* Bunge., *A. viciaefolius* DC., and *A. virgatus* Pall. Apparently there is no specialization of forms within this species of *Uromyces*. Aecidiospores from *Euphorbia virgata* W.K. and *E. Gerardiana* Jacq. infected *Caragana frutescens* DC. (*Uromyces Genistae-tinctoriae* [Pers.] Fuckel). Other leguminous plants, including *Astragalus* and *Medicago*, were not infected either by aecidiospores or by uredospores. Aecidiospores from another aecidium on *Euphorbia virgata* infected *Medicago falcata* (*Uromyces striatus* Schroet.), but not *Astragalus*, *Caragana*, and others. Aecidiospores from *Cichorium Intybus* L. infected *Juncus Gerardi* Lois (*Puccinia Junci* [Strauss] Winter). A sowing of aecidiospores from *Taraxacum serotinum* W.K. produced only uredospores, probably those of *Puccinia silvatica* Schroet., on *Carex stenophylla* Wahlenb. Uredospores of *Puccinia Cesatii* Schroet. on *Andropogon Ischaemum* L., which had persisted during an unusually severe winter, were capable of germinating and infecting that host in spring. Apparently this rust lives through the winter in that region by means of uredospores which have thick walls. Teleutospores of *Puccinia Stipae* (Opiz.) Arthur from *Stipa Lessingiana* Trin. infected *Salvia aethiopis* L., *S. nutans* L., *S. silvestris* L., *Thymus serpyllum* L., and *Ajuga chia* Schreb.

STRELIN,<sup>19</sup> in a series of cultures, shows the relation between the primary uredinia (*Uredo Muelleri* Schroet.) and secondary uredinia and telia of *Kuhnneola albida* (Kühn) Magnus, confirming the work of JACKY showing that these spore forms all belong to the same rust. STRELIN also shows that the fungus persists through the winter by means of the spores of the primary uredinia which are formed in July and August. The primary uredospores can infect the following spring only the old leaves which have persisted through the winter.—H. HASSELBRING.

<sup>18</sup> TREBOUX, O., Infektionsversuche mit parasitischen Pilzen. I. Ann. Myc. 10:73-76. 1912.

<sup>19</sup> STRELIN, S., Beiträge zur Biologie und Morphologie der *Kuhnneola albida* (Kühn) Magn. und *Uredo Muelleri* Schroet. Mycol. Centralbl. 1:92-96, 131-137. 1912.

**Cecidology.**—Among the most important of the foreign contributions is a paper by KARNY,<sup>20</sup> in which the author describes a large number of gall-making *Thysaneura*, including a number of new species. However, the emphasis is placed upon the animal rather than the plant side of the subject.

A most excellent paper by HOUARD<sup>21</sup> on the galls of French West Africa gives descriptions of 51 new species of gall-makers on 29 species of host plants. The author describes both insect and cecidia and in most cases gives illustrations of the cecidia. The grouping is with reference to the plants, a practice which is apparently growing in favor of the students of cecidology.

Root nodules are the subject of two papers. Miss SPRATT<sup>22</sup> discusses the root nodules on the Podocarpaceae which are caused by *Pseudomonas radicola*. These nodules are modified lateral roots. The organism penetrates the root-hairs and then the cortex, and the stimulus is in the meristematic tissue, but there is no differentiation of the meristematic zone in the cortical tissue such as is found in the nodules of other non-legumes. The organism produces a zooglea in the cells which stimulates the nuclei of the host and causes them to divide amitotically. In the spring the cells immediately below the endodermis at the apex of the nodular stele become meristematic and produce new cortical cells in the interior of the old nodes. The other paper on root nodules is by BOTTOMLEY,<sup>23</sup> who finds the nodules on the *Myrica Gale* are also modified lateral roots. These nodules are also caused by *P. radicola* and each mature nodule shows four zones: (a) the apical meristem, (b) the infected thread area, (c) the bacterial zone, (d) the basal zone in which the cells contain oil drops. The bacteria eventually disappear and the basal zone is replaced by the other zones.

A very important contribution to our knowledge of American cecidology is BESSEY'S<sup>24</sup> study of the nematode root knots. This disease is caused by *Heterodera radicola* (Greef) Mull., and was probably indigenous in some tropical region of the Old World from which it has been distributed throughout the tropics and a considerable part of the temperate zones. Records show that about 480 species and varieties of plants, including nearly all the larger families, are subject to this disease. The life cycle is four weeks or more, dependent on the temperature of the soil. The author gives a good discussion

<sup>20</sup> KARNY, K. Gallenbewohnende Thysanopteren aus Java. Marcellia 11:115-169. 1912.

<sup>21</sup> HOUARD, C., Les galles de l'Afrique occidentale française (V. *Cecidies nouvelles*). Marcellia 11:176-209. 1912.

<sup>22</sup> SPRATT, ETHEL ROSE, The formation and physiological significance of root nodules in the Podocarpaceae. Ann. Botany 26:803-813. 1912.

<sup>23</sup> BOTTOMLEY, W. B., The root nodules of *Myrica Gale*. Ann. Botany 26:111-117. 1912.

<sup>24</sup> BESSEY, E. A., The root knot and its control. U.S. Bur. Pl. Industry, Bull. 217. 1911.

of the life history of the organism, methods of distribution, and methods of control.

The potato eelworm is the subject of a brief paper by ESSIG.<sup>25</sup> He describes the disease and the organism and suggests treatments. The disease is of such great importance that California has established a quarantine against potatoes from infected districts.

A recent importation from Europe is reported by CRAWFORD.<sup>26</sup> It is *Trioxa alacris* Flor., which causes a rolling and distorting of the leaves of *Laurus nobilis* and other species of *Laurus*.

The olive knot is the subject of a very interesting paper by HORNE.<sup>27</sup> This disease is restricted to the olive, and is due to *Bacterium savastanoi* E. F. Smith. It causes knots very similar to the crown gall, but restricted entirely to the branches. It is distributed by means of a slime which oozes from the galls during the rainy weather. Inoculation may occur through natural cracks in the bark.

FAWCETT<sup>28</sup> describes some very interesting citrus galls from Southern California. He believes them to be different from the Jamaica lime and orange knot, which is due to *Sphaeropsis tumefaciens* Hedges.

One of the most interesting cecidia is reported by AMUNDSEN,<sup>29</sup> who found it on wistarias imported from Japan. It occurs on the stems at the base of the buds and in most cases causes the death of the affected buds. Only the pink-flowered plants were infected, and in commenting on this point the author says "whether the fly which laid the eggs discriminated against all the plants which ultimately would produce flowers of other colors and could pick out the pink, or whether the pink varieties were grown in a different locality than the others, could not be ascertained and is still a mystery."

HOUSER<sup>30</sup> describes a gooseberry gall as follows: "The plant is injured by the insect working during the larval stage in the terminal buds of spurs and branches, causing the buds to become abnormal both in size and structure. The bud scales increase greatly in number and size, and lying closely one upon another form a gall somewhat resembling in miniature the pine cone willow galls so commonly encountered upon the tips of willow twigs." Secondary buds are produced and become infected, thus forming a cluster of small cone-shaped galls. The shoots from these galls give a witches' broom effect. The

<sup>25</sup> ESSIG, E. O., The potato eelworm. Monthly Bull. State Comm. Hort. (Cal.) 1:26-30. 1911.

<sup>26</sup> CRAWFORD, D. L., A new insect pest. *Op. cit.* 1:86, 87. 1912.

<sup>27</sup> HORNE, W. T., The olive knot. *Ibid.* 1:592-600. 1912.

<sup>28</sup> FAWCETT, H. S., Citrus galls. *Ibid.* 1:937-940. 1912.

<sup>29</sup> AMUNDSEN, E. O., Wistaria gall fly. *Ibid.* 1:730-733. 1912.

<sup>30</sup> HOUSER, J. S., The gooseberry gall midge or bud deformer. Jour. Econ. Entomol. 5:180-184.

author also describes the insect, gives its life history, and suggests methods of control.—MEL. T. COOK.

**Paleobotanical notes.**—Miss HOLDEN<sup>31</sup> has investigated specimens of a conifer from the Trias of New Brunswick which she refers to *Volzia coburgensis* Schaur. She finds that the foliage of this species is araucarian, the organization of the cone abietineous, and the anatomical structure intermediate between these two groups. This species of *Volzia*, therefore, represents another early mesozoic form which may be regarded as transition from the Abietineae to the Araucarineae.

Miss BANCROFT<sup>32</sup> has added to the evidence of the uniformity of the mesozoic floras in describing some fossil gymnosperms obtained from the Jurassic of India. The cycadophyte remains are of the *Williamsonia* type, and the vegetative organs show a combination of the characters of Bennettitales and Cycadales.

Miss BANCROFT<sup>33</sup> has described a new stem genus (*Rhexoxylon*) from the later Paleozoic of South Africa. Its general structure suggests relationship with *Medullosa* and *Steloxylon*, and it seems certainly to be an addition to the Medulloseae. A peculiar feature is the character of the inner series of vascular strands, each one consisting of two parts, the outer small and normally oriented, the inner larger and inversely oriented, the two parts being almost in contact. An outer series of strands consists of normally oriented xylem. As contrasted with medullosean stems in general, the wood is compact, the medullary rays are uniseriate, and the pitting of the tracheids is biseriate.

SEWARD and Miss BANCROFT<sup>34</sup> have added to the list of species of Scottish Jurassic plants, describing new species in *Thinnfeldia*, *Brachyphyllum*, *Masculostrobilus*, *Conites*, *Strobilites*, and *Cedroxylon*.

SEWARD<sup>35</sup> has published a memoir dealing with two collections of mesozoic plants: the principal one from Afghanistan, made by Mr. H. H. HAYDEN in 1907; the other from Turkistan, made by Mr. GRIESBACH. The composition of the Afghanistan flora is interesting, including the following great groups: Equisetales (several species of *Equisetites*), Filicales (one water fern and several true ferns, including a new genus, *Haydenia*, of Cyatheaceae), Ginkgoales (3 genera), Bennettitales (7 species, among them a new *Williamsonia* and a new *Nilssonio*),

<sup>31</sup> HOLDEN, RUTH, Some fossil plants from Eastern Canada. *Ann. Botany* 27: 243-255. pls. 22, 23. 1913.

<sup>32</sup> BANCROFT, NELLIE, On some Indian Jurassic gymnosperms. *Trans. Linn. Soc. London II. Bot.* 8: 69-86. pls. 7-9. 1913.

<sup>33</sup> BANCROFT, NELLIE, *Rhexoxylon africanum*, a new medullosean stem. *Ibid.* 87-103. pls. 10, 11. 1913.

<sup>34</sup> SEWARD, A. C., and BANCROFT, N., Jurassic plants from Cromarty and Sutherland, Scotland. *Trans. Roy. Soc. Edinburgh* 48: 867-888. pls. 1, 2. 1913.

<sup>35</sup> SEWARD, A. C., Mesozoic plants from Afghanistan and Afghan-Turkistan. *Mem. Geol. Surv. India N.S.* 4: no. 4. pp. 57. pls. 7. 1912.

5 species of *Podocarpites* (3 of which are new), and Coniferales (5 species, among them a new *Cupressinoxylon*).

SEWARD,<sup>36</sup> in reporting upon a collection of fossil plants from the Wealden of Sussex, describes new species in *Lycopodiites*, *Selaginellites*, *Hausmannia* (Dipteridineae), *Pelletieria* (a new genus of Schizaeaceae), *Teilhardia* (a new genus of ferns of uncertain affinity), *Dichopteris*, *Conites* (cones of uncertain affinity). In a general survey of the Wealden floras, the author concludes that "while there is a very close similarity between the Wealden flora of England and the corresponding floras in Eastern and Western North America, the number of cosmopolitan types is smaller than in the case of the Middle Jurassic floras."—J. M. C.

**Chondriosomes and myelin forms.**—The problem of the chondriosomes, or mitochondria, is approached from a new direction in a short article by LÖWSCHIN<sup>37</sup> who happened to notice the formation of myelin forms<sup>38</sup> from lecithin in a microscopic preparation. These myelin forms bore such a remarkable resemblance to chondriosomes that LÖWSCHIN made a careful examination of myelin forms secured from commercial lecithin. The following are some of the more important results: all the forms characteristic of chondriosomes were obtained and their size varied from structures easily seen with a low-power dry objective to those barely visible under the highest powers. In general, the size depends upon the mass of the material, the fineness of its division, and the chemical and physical characteristics of the surrounding medium; while the form is dependent upon the composition of the bodies, their surface tension, and the nature of the surrounding medium. It is to be noted that the elongated forms are found when there is streaming in the surrounding medium. The myelin forms may appear homogeneous or may show a finer structure, and the outer membrane may be liquid or may have more consistency. In many cases a longitudinal splitting, like that described by LEWITSKY for chondriosomes, was observed. The myelin forms arise, develop, and disappear. They may swell and flow together, forming homogeneous threads (*Chondriokonten*), from which are developed granular threads (*Chondriomiten*), which may then break up into single granules (mitochondria), and these again may form into chains. One can observe directly the formation of diplosomes and their division into two granules. The myelin forms, like chondriosomes, may be fixed by chromic acid, osmic acid, or formalin, but are destroyed by acetic acid.

<sup>36</sup> SEWARD, A. C., Contribution to our knowledge of Wealden floras. Quart. Jour. Geol. Soc. 69:85-116. pls. 11-14. 1913.

<sup>37</sup> LÖWSCHIN, A. M., Myelinformen und Chondriosomen. Ber. Deutsch. Bot. Gesells. 31:203-209. 1913.

<sup>38</sup> By "myelin forms" is meant the emulsion forms which, under the action of emulsion-producing substances, form upon fatty acids.

While Löwschin admits that at present he is describing analogies, still he believes that these are too numerous and too striking to be merely accidental.—CHARLES J. CHAMBERLAIN.

**Recent work among gymnosperms.**—SAXTON<sup>39</sup> has investigated one of the two species of *Acinostrobos*, an endemic Australian genus, and therefore well worth investigation. An outline of the results is as follows. The microsporophyll bears three sporangia and about three months elapse between pollination and fertilization. The archegonia are numerous and deep-seated, "a group of 25-30 being found abutting on the lower end of each pollen tube, which reaches about halfway down the prothallus," the older cells of which are generally 2-nucleate or 4-nucleate. In proembryo-formation, walls are formed when the two free nuclei divide, so that there is a 4-celled proembryo. The completed proembryo, consisting of few cells, fills the egg. Each cell of the proembryo (with perhaps the exception of the two "apical cells") gives rise to a suspensor and an embryo-initial, being as independent in embryo-formation as are the proembryonal cells of *Ephedra*. The chromosome numbers are 8 and 16.

TAKEDA<sup>40</sup> has studied in detail the anatomy of the leaf of *Welwitschia* and concludes that the evidence is all in favor of the Gnetales being gymnosperms, as opposed to the view of LIGNIER and TISON. Even the tracheae, the most striking angiospermous anatomical feature, are in a transition stage, showing incomplete perforations.

TAKEDA<sup>41</sup> has developed a theory of the so-called "transfusion tissue" of gymnosperms. He finds that the "orthodox" transfusion tissue always arises laterally, and is quite independent of centripetal xylem. Therefore, it is not a vestige of the centripetal xylem and is not to be regarded as of phylogenetic significance, its function being "water-storing."—J. M. C.

**Gemmae in *Radula*.**—The development of gemmae in two species of *Radula* has been studied by Miss WILLISTON.<sup>42</sup> In *R. flaccida*, a native of tropical America, the gemmae occur on the dorsal margin of the leaves, and formation begins by the enlargement of a single cell around which a transparent gelatinous substance is secreted. A periclinal wall divides the gemma initial into a stalk cell which undergoes no further division, and an outer or mother cell which is divided by an anticlinal wall. The next division gives a quadrant, the two outer cells of which immediately function as apical cells with two cutting faces. The two inner cells of the quadrant do not produce apical cells.

<sup>39</sup> SAXTON, W. T., Contributions to the life history of *Acinostrobos pyramidalis* Miq. Ann. Botany 27:321-345. pls. 25-28. 1913.

<sup>40</sup> TAKEDA, H., Some points in the anatomy of the leaf of *Welwitschia mirabilis*. Ann. Botany 27:347-357. pl. 29. 1913.

<sup>41</sup> TAKEDA, H., A theory of "transfusion tissue." Ann. Botany 27:359-363. 1913.

<sup>42</sup> WILLISTON, RUTH, Bull. Torr. Bot. Club 39:329-339. figs. 37. 1912.



The gemmae at maturity measure 0.5 mm. in diameter. The adult leaf measures 0.8 mm. in diameter. In *R. protensa*, a native of New Guinea and adjacent regions, the initial becomes covered with a greater quantity of gelatinous material than in *R. flaccida*. As the gemma increases in size it finally bursts through the gelatinous covering, which then clings to the base like a collar.

The gemmae of *Radula* are arranged in two groups according to complexity. In the first group the gemmae occur on margins of leaves, are irregular in outline when mature, and may be more than one cell thick; in the second they occur on the margin and surface of leaves, are regular in development and symmetrical in form, and are only one cell thick. *R. flaccida* and *R. protensa* belong to the second class.—W. J. G. LAND.

**Cytology of Hymenomycetes.**—LEVINE,<sup>43</sup> working in Harper's laboratory, has investigated the carpophores of 24 species of *Boletus* and of several species of *Polyporus*, has had in cultures the mycelia of various Hymenomycetes, and has secured some spore germination (none of the spores of *Boletus* germinated), so that his observations of the nuclear phenomena are somewhat extensive. The germinating spores of *Pholiota praecox* produce multinucleate germ tubes; in cultures 48 hours old the cells of the mycelium are multinucleate; but in cultures 3 days old, both uninucleate and binucleate cells are found. The mycelial cells of many species are binucleate, with clamp connections, etc. In the mature stipe of *Boletus granulosus* all the cells are multinucleate; while those of the ring, of the flesh and trama, and of the subhymenium are binucleate. At the end of the second division of the fusion nucleus in the basidium, the centrosomes become attached to the walls of the basidium and the 4 daughter nuclei remain connected with them by fibrillar strands. The centrosomes determine the points of origin of the 4 sterigmata and are carried up with the growth of the sterigmata and into the spores, pulling the nuclei into the spores. All the spores studied were uninucleate at first. The conclusion is that an alternation of generations "comparable to that in the Uredineae" is also present in these forms. "The sporophyte begins at some indefinite point in the mycelium and extends through the development of the carpophore."—J. M. C.

**Aluminium salts.**—FLURI<sup>44</sup> has claimed that aluminium salts render certain plant cells incapable of being plasmolyzed by ordinary plasmolytic agents by rendering the protoplasm highly permeable to these reagents. SZÜCS<sup>45</sup> finds that the protoplasm is rendered less permeable to many agents by aluminium salts,

<sup>43</sup> LEVINE, MICHAEL, Studies in the cytology of the Hymenomycetes, especially the Boleti. Bull. Torr. Bot. Club 40:137-181. pls. 4-8. 1913.

<sup>44</sup> BOT. GAZ. 47:252. 1909.

<sup>45</sup> SZÜCS, JOSEPH, Über einige charakteristische Wirkung des Aluminiumions auf das Protoplasma. Jahrb. Wiss. Bot. 52:269-332. 1913.

and that the incapacity for plasmolysis is due to a hardening of the protoplasm. The main evidence for the hardening of the protoplasm is the failure of chloroplasts to be displaced by a centrifugal force of about 1000 gravities, which ordinarily displaces them readily. This process of hardening is reversed (physico-chemically and physiologically) by transferring the cells to aluminium-free solutions. The slow rate of reversing leads the author to postulate the tying up of the aluminium in an indiffusible form. It is only in moderate concentrations that aluminium salts produce the effect; in higher concentrations the protoplasm does not harden. Anthocyanin cells cannot be thus hardened, probably due to high sugar content. The author relates this to the fact that non-electrolytes hinder the precipitation of proteins by ions. He also thinks that aluminium salts are specific only in that their low toxicity enables them to bring about such a fundamental physical change in the protoplasm without killing it. While most salts produce such a change, it is not physiologically reversible.—WILLIAM CROCKER.

**Individuality of the chromosome.**—A four-years' study of the vegetative and reproductive nuclei of *Carex aquatilis* has brought STOUT<sup>46</sup> to the conclusion that the chromosome is an individual organ, maintaining its identity through successive cell generations. He finds that even in resting nuclei the chromosomes are visible as definite bodies which can be counted, and that these chromosomes can be traced through all the stages of vegetative and reduction divisions, except synapsis. Spiremes are formed in both vegetative and reduction divisions, but even in the spirem the individual chromosomes are distinguishable. In the vegetative spirem there is no evidence of any splitting, the longitudinal splitting of the chromosome appearing only after the chromosomes have become arranged in the nuclear plate. In the metaphase of the heterotypic mitosis the chromosomes form a more or less obvious double spirem. The heterotypic mitosis separates whole chromosomes which have been previously paired. Although the study would have been more satisfactory if the stages in synapsis had been more complete, the results form an important addition to the already strong evidence that the chromosome is an individual organ. *Carex aquatilis*, like *Carex acuta* described by JUEL, forms only one pollen grain from a pollen mother cell, the other three aborting at an early stage.—CHARLES J. CHAMBERLAIN.

**Seedling anatomy.**—HILL and DEFRAINE<sup>47</sup> have worked long enough upon seedling anatomy to have come to some very interesting conclusions. Citing numerous facts that have been used in phylogenetic conclusions, they state that they "see no necessity for preserving seedling anatomy from the fate already meted out to other structural features which were at one time considered as

<sup>46</sup> STOUT, A. B., The individuality of the chromosomes and their serial arrangement in *Carex aquatilis*. Archiv. Zellforschung 9:114-140. pls. 11, 12. 1912.

<sup>47</sup> HILL, T. G., and DEFRAINE, E., A consideration of the facts relating to the structure of seedlings. Ann. Botany 27:257-272. 1913.

indicators of phylogeny." "In fact, until more knowledge is obtained with regard to the interrelationship of plant members and the influence of environment—in a word, the influence of physiological necessity on morphological expression—we cannot determine with any degree of certainty the precise value of many anatomical characters." The same disposition is made of the size and number of vascular bundles in connection with the transition phenomena. "Sufficient has been said to show the enormous importance of physiology in questions relating to vascular tissues; for our own part we are strongly of the opinion that no real further advance in our knowledge of morphology, more especially of the higher plants, is possible without an adequate investigation of the physiology of the members concerned."—J. M. C.

**Inheritance of quantitative characters.**—EMERSON and EAST<sup>48</sup> have discussed this subject rather fully and have presented data bearing upon it secured from experiments with maize. Inheritance was studied in number of rows per ear, length of ear, diameter of ear, weight of seeds, breadth of seeds, and height of plants. The general conclusion is stated compactly as follows: "The results secured in the experiments with maize were what might well be expected if quantitative differences were due to numerous factors inherited in a strictly Mendelian manner. It is quite likely that genetic correlations occur between factors for distinct quantitative characters. These and the physiological correlations so frequently noted make the results more difficult of interpretation, but do not throw them out of the realm of Mendelian phenomena. Physiological correlation is a phenomenon of development, not of inheritance, and as such has less interest for students of genetics than for experimental morphologists. Even in practical plant breeding, correlations of this sort are of importance mainly on account of the physiological or morphological limits that they set to the perfect development of particular combinations of characters."—J. M. C.

**The individuality of the plastid.**—In a preliminary paper<sup>49</sup> published two years ago, Sapehin found plastids even in sporogenous tissue, both in monoplasic types, like *Anthoceros*, *Isoetes*, and *Selaginella*, and in polyplastic forms, like the majority of plants. In a second preliminary account<sup>50</sup> he deals principally with *Lycopodium*, which he finds to belong to the monoplasic type; and with *Funaria*, which belongs to the polyplastic type. In the antheridium of *Funaria*, which starts as a polyplastic organ, cell division is not accompanied by any division of the plastid, and consequently the spermatogenous cells soon become

<sup>48</sup> EMERSON, R. A., and EAST, E. M., The inheritance of quantitative characters in maize. Agric. Exper. Station, Univ. Neb., Research Bull. 2. pp. 120. figs. 21. 1913.

<sup>49</sup> SAPEHIN, A. A., Über das Verhalten der Plastiden in sporogenen Gewebe. Ber. Deutsch. Bot. Gesells. 29:491-496. figs. 5. 1911.

<sup>50</sup> ———, Untersuchung über die Individualität der Plastide. Ber. Deutsch. Bot. Gesells. 31:14-16. 1913.

monoplastic; each sperm receives one plastid. SAPÉHIN claims that the bodies described by ALLEN, in the spermatogenesis of *Polytrichum*, as blepharoplasts, are nothing but plastids. Since SAPÉHIN's account is not illustrated, one must feel rather skeptical in regard to his interpretation of ALLEN's work.

—CHARLES J. CHAMBERLAIN.

**Botrychium Lunaria.**—LANG<sup>51</sup> has published the first of a series of papers on the vascular anatomy of the Ophioglossaceae. From examination of *Botrychium Lunaria* he concludes that the internal endodermis is a new formation without morphological significance. The occurrence of occasional tracheids in the pith, especially in injured plants, is accepted as conclusive evidence of the stelar nature of the pith. These scattered tracheids are primary in their origin and by reason of their position are identified as centripetal xylem; hence this species of *Botrychium* has mesarch stem structure.

Axillary buds are of constant occurrence in this species, as in *Helminthostachys*. These develop only in case of injury to the terminal bud. The vascular supply of the branch arises from the trace of the leaf immediately below by the development of adaxial xylem; occasionally the xylem of the branch may be connected directly with that of the main stem.—L. C. PETRY.

**Lichens of the Galapagos Islands.**—STEWART<sup>52</sup> has reported on the lichens collected by the expedition of the California Academy of Sciences to the Galapagos Islands in 1905-1906. These islands have long been of great biological interest, so that any collection from them promises to be worth noting. The list numbers 47 species, 7 of which are undetermined. The genera represented are as follows: *Alectoria* (1 sp.), *Arthonia* (4 spp.), *Buellia* (2 spp.), *Chiodecton* (1 sp.), *Cladonia* (6 spp.), *Coenogonium* (1 sp.), *Lecanora* (3 spp.), *Lecidea* (1 sp.), *Pannaria* (1 sp.), *Parmelia* (4 spp.), *Pertusaria* (2 spp.), *Physcia* (2 spp.), *Placodium* (1 sp.), *Pyrenula* (1 sp.), *Ramalina* (5 spp.), *Rinodina* (1 sp.), *Roccella* (2 spp.), *Sticta* (2 spp.), *Teloschistes* (1 sp.), *Usnea* (4 spp.), *Verrucaria* (1 sp.).—J. M. C.

**Plants of northwestern Canada.**—STANDLEY<sup>53</sup> has determined the plants collected by an expedition to the Mount Robson region of eastern British Columbia and western Alberta in 1911, under the auspices of the Alpine Club, whose headquarters are at Banff, Alberta. The collection includes about 200 numbers, all of which are angiosperms except 5 mosses, 6 pteridophytes, and 3 gymnosperms. A new species was discovered in each of the following genera: *Carex*, *Vagnera*, *Artemisia*, *Aster*, *Gaillardia*.—J. M. C.

<sup>51</sup> LANG, WILLIAM H., Studies in the morphology and anatomy of the Ophioglossaceae. I. On the branching of *Botrychium Lunaria*, with notes on the anatomy of young and old rhizomes. Ann. Botany 27:203-242. figs. 1-14. pls. 20-21. 1913.

<sup>52</sup> STEWART, ALBAN, Notes on the lichens of the Galapagos Islands. Proc. Calif. Acad. Sci. IV. 1:431-446. 1912.

<sup>53</sup> STANDLEY, PAUL C., Plants of the Alpine Club expedition to the Mount Robson region. Canadian Alpine Journal, special number, pp. 76-97. 1912.

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CAN FUNGI LIVING IN AGRICULTURAL SOIL ASSIMILATE FREE NITROGEN?

H. N. GODDARD

(WITH EIGHTEEN FIGURES)

I. Historical introduction

As early as the middle of the nineteenth century it was known to BOUSSINGAULT (cited by SACHS 1) that plants cannot make use of atmospheric nitrogen in their nutrition, but are dependent on combined nitrogen which they ordinarily get from the soil. This idea has become so thoroughly established in all later works that any evidence of nitrogen-fixation by plants is received with unusual interest. Such evidence has come almost entirely from studies of lower organisms, such as bacteria, algae, and fungi.

The full establishment of the nitrogen-fixing power of certain bacteria calls for little discussion in this paper. The important and now classical studies of HELLRIEGEL (2), BEYERINCK (3), HILTNER (4), WINOGRADSKI (5), and STOKLASA (6) have put on a sure scientific basis the fact that a number of bacteria, including not only those associated with root tubercles, but several others, have the power to assimilate free nitrogen, especially when deprived of sufficient quantities in the combined form. It is perhaps worthy of note, also, that this power has been found in many cases to be much augmented by a symbiotic relationship with other organisms, such as algae, Leguminosae, and with other bacteria. A good résumé of the literature of this subject has been given by CHESTER (7), KOCH (8), and HEINZE (9).

With this well established knowledge regarding bacteria, the suggestion has been easy that the closely related organisms, the fungi, might also be found, at least some of them, to possess a similar nitrogen-assimilating power. Acting on this suggestion, a number of investigators within the last 15 years have given attention to this phase of the problem.

Inasmuch as this paper is concerned mainly with this question, a brief historical survey seems desirable. The oldest work on nitrogen-fixation is cited by FRANK (10), and later by FROELICH (11), as having been carried on by JODIN (12) in 1862. A rich fungous growth was observed on nitrogen-free media supplied with sugar, tartaric acid, or glycerin. The purity of the cultures was not certain, although atmospheric nitrogen compounds were excluded and analytical methods were used. Later, BERTHELOT (13) in 1893 reported nitrogen-fixation for three fungi: *Aspergillus niger*, *Alternaria tenuis*, and a species of *Gymnoascus*, cultivated on a medium containing COHN's (cited by SMITH 14) solution, to which had been added tartaric acid or sugar and kaolin. He claims purity of culture only for *Alternaria*, in which case he reports a nitrogen gain as high as 98 per cent of the original nitrogen content. During the same year, FRANK (15) also reported nitrogen-fixation in certain fungi which he found growing on a nitrogen-free solution of sugar and mineral constituents. Later, using *Penicillium cladosporioides* Fres. (*Hormodendron cladosporioides* Sacc.) on a similar solution, and applying chemical analysis to determine nitrogen gain, he found a gain of 3.5 mg. in a 65 cc. culture.

Further positive results have more recently been reported by PURIEWITSCH (16), SAIDA (17), TERNETZ (18), FROELICH (11), and LATHAM (20). PURIEWITSCH, using *Aspergillus niger* and *Penicillium glaucum*, cultivated on nitrogen-free media with tartaric acid and varying quantities of cane sugar, secured a nitrogen gain of 1.5-8.4 mg. for *Aspergillus* and 2.0-5.2 mg. for *Penicillium*. SAIDA worked with a large number of species, including *Phoma betae*, *Mucor stolonifera*, *Aspergillus niger*, *Endococcus purpurascens*, *Acrostalagmus cinnabarinus*, *Monilia variabilis*, and *Fusisporium moschatum*. He cultivated these on synthetic media with varying quantities of sugar. He also used cultures with and without fixed

nitrogen. In the first four species, he found a nitrogen gain, running as high in one case as 10.53 mg. in a 50 cc. culture of beet sugar decoction. The last three species showed negative results throughout.

TERNETZ first isolated a pycnidium fungus from the roots of *Oxycoccus*, reporting that it had the power of fixing free nitrogen to the amount of 6-10 mg. per 1 gm. of dextrose used. Later, the same author (19) contributed another paper on other pycnidia fungi, isolated from the roots of Ericaceae and referred to the genus *Phoma*. These were cultivated on nitrogen-free media based on WINOGRADSKY'S formula, but using varying quantities of sugar, phosphate, and magnesium. In a full and painstaking investigation, the author reports free nitrogen assimilated by the 5 species of *Phoma* as follows: 2.17, 3.99, 10.9, 18.0, 22.1 mg. per 1 gm. of dextrose used. In the same investigation, *Aspergillus niger* and *Penicillium glaucum* were reported to gain 1.71 and 3.8 mg. respectively per 1 gm. of dextrose used.

Four years later, FROELICH contributed a careful study of four fungi isolated from decaying stems and leaves. The forms were identified as *Alternaria tenuis* Nees, *Macrosporium commune* Rbh., *Hormodendron cladosporium* Sacc., and *Cladosporium herbarium* Link. These were cultivated on a medium similar to WINOGRADSKY'S for *Clostridium pasteurianum*, with quantities of dextrose varying from 2 to 5 gm. per 100 cc., and the cultures were aerated by air freed from combined nitrogen. The author reports nitrogen gains of 2.56-8.92 per 1 gm. of dextrose used. FROELICH also reported confirmation of TERNETZ'S results on *Aspergillus niger* and *Penicillium glaucum*, although much smaller gains are reported by FROELICH.

Perhaps the latest positive results for nitrogen-fixation by fungi have been contributed by LATHAM (20), who worked on *Sterigmacystis nigra* (*Aspergillus niger*), cultivated in solutions containing ammonium nitrate, potassium dihydrogen phosphate, magnesium sulphate, sugar, and a trace of iron. Some of the cultures were modified by the addition of zinc sulphate, but this was reported to have an inhibiting effect on the nitrogen-assimilating power. The cultures without zinc sulphate are reported to fix nitrogen

in quantities all the way from 1.6 to 205.1 mg. The author believes that the critical point of the fungus with regard to the nitrogen supply is slightly below 160.3 mg. of nitrogen in 50 cc. of the solution, and when a greater quantity of nitrogen is supplied to the fungus, it ceases to fix nitrogen and begins to consume it.

All this weight of evidence would seem to establish fully the fact of nitrogen-fixation by fungi, were there not also a large and increasing amount of negative evidence by well recognized investigators. WINOGRADSKY (21) has reported that he failed to confirm the results of BERTHELOT on *Aspergillus niger*, while KOCH (8) states that he was unable to confirm the work of either PURIEWITSCH or SAIDA, giving as his opinion that they worked with impure cultures or were in some other way mistaken in their results. Again, CZAPEK (22) reports negative results for *Aspergillus niger*. Also HEINZE (9) has recorded a repetition of the work of PURIEWITSCH and SAIDA, using similar solutions as well as numerous modifications, including variation of the sugar content and also of the fixed nitrogen content. In no case was he able to confirm the earlier work. He fails, however, to give his analytical data. Further, it may be noted that TERNETZ disagrees quite widely with PURIEWITSCH regarding the amount of nitrogen gain in the case of *Penicillium* and *Aspergillus* in nitrogen-free solutions. It may be added further that BREFELD (23) and HEINZE (9) have reported negative results with reference to the nitrogen-fixing power of certain species of *Ustilago* and with *Dematium*-like fungi and yeasts.

More recently, further weight has been added to the negative side by the work of PENNINGTON and DUGGAR, as well as by the work of this investigation. PENNINGTON (24) first reported no nitrogen-assimilating power for a species of *Fusarium* isolated from the soil. Later (25) he reports absolutely no nitrogen-fixing power for *Aspergillus niger*, *Penicillium glaucum*, and *Alternaria* (sp.), either in nitrogen-free or in nitrogen-containing media, using methods similar to those of FROELICH and TERNETZ. DUGGAR (26) has very recently reported negative results for the following fungi: *Coprinus comatus*, *Daedalea quercina*, *Polyporus sulphureus*, *Trichoderma lignicola*, and *Aspergillus niger*. With these he carried



out two series of experiments, involving about 400 flask cultures with a great variety of culture media. Analyses showed that in no case was there fixation of atmospheric nitrogen, except possibly in certain cultures of *Aspergillus niger*. In many cases, DUGGAR reports there was a loss of nitrogen, which he attributes, usually at least, to the production of gaseous nitrogen. A third series of experiments is referred to, in which additional fungi have been tested and the experiments of other investigators have been duplicated with negative results.

It is beyond the province of this paper to consider the mycorrhiza fungi, any more than merely to refer to the work of HILTNER (27), FRANK (28), and others, who have reported nitrogen-fixation by such fungi.

Discussion of these apparently very conflicting results will be reserved till a later part of this paper, the purpose here being only to give a brief historical review of the work on this problem up to the present time.

The investigation described in this paper was begun several years ago, under the direction of Professor J. B. POLLOCK at the University of Michigan. The following purposes were in mind: (1) to determine what species of fungi live habitually in an ordinary agricultural soil; (2) to study their distribution as to depth and nature of the soil; and (3) to begin some study of the part they play in soil fertility. After isolating and making a study of about 17 species, attention was turned to the problem of nitrogen-fixation, since it was thought, from our knowledge of bacteria, that if any fungi possessed nitrogen-fixing power, such forms might well be looked for in the soil. Furthermore, if any soil fungi were found to fix nitrogen to any marked degree, such a fact would have important bearing on soil fertility, as well as a large interest from the purely physiological standpoint.

## II. Isolation and identification of soil fungi

### HISTORY OF SOIL FUNGI INVESTIGATIONS

Although many of the fungi living in the soil have been known for some time, and many attempts have been made to isolate mycorrhiza forms, little work seems to have been done previously

in studying the fungous flora of the soil as such. This is the more surprising, since what study has been carried on indicates that there is a very abundant and distinct group of forms which live habitually in the soil.

Perhaps the most extensive work in isolating and studying these forms is that of OUDEMANS and KONING (29), who reported 45 species from a humous soil of the forest of Spanderswoud, near Bussum, in Holland. It seems quite remarkable that of these 45 species, 32 were named as new species which inhabit the soil. These belonged to the Hyphomycetes and Phycomycetes. Few other similar studies seem available, although ADAMETZ (30) has carried on a study of organisms found in a field soil. He reports having isolated, besides a number of bacteria and yeasts, the following fungi: *Penicillium glaucum*, *Mucor mucedo*, *M. racemosus*, *M. stolonifer*, *Aspergillus glaucus*, and *Oidium lactis*. Again, HAGEM (31) has recently reported an investigation of Mucorineae which he isolated from soil and air; 16 species were obtained from the soil, and it is interesting to note that he named 8 of these as new species.

Since the completion of this investigation, and after the appearance of a preliminary paper by the author (Proc. Mich. Acad. Sci. 1911), a paper by DALE (46) on soil fungi has been received. This investigator reports and figures 20 genera of fungi isolated from a sandy soil obtained from the Royal Agricultural Society's Farm at Woburn, England. The resemblance between DALE's list and that of this paper is certainly very striking. Not only many of the genera, but a number of the species, are the same. I note also in DALE's paper a reference to a recent paper by JENSEN (47). It is evident from these numerous recent studies that much attention is being given to this subject.

#### SELECTION OF A SOIL PLAT

For the isolations of this investigation, a plat of rather rich, clay loam was selected from a garden in Ann Arbor. It had been in use for many years for raising common garden vegetables, usually in rotation. As a rule, it had been heavily manured, but during the last few seasons this had been less frequent and less

abundant. No manure had been used the year previous, although a garden crop had been raised. The entire plat was 20×60 feet. This was divided into three equal squares which were treated as follows: plat I was untilled and unfertilized; plat II was well tilled but unfertilized; while plat III was both well tilled and well fertilized with stable manure. Samples of the soil from these plats were taken in a manner hereafter described, and cultures were made in the laboratory by the usual method, outlined by DUGGAR (32, pp. 34-40). Pure cultures were then isolated and studied.

#### CULTURE MEDIA

Media for the platings and pure cultures were chosen with reference (1) to the idea of inhibiting the growth of bacteria, and (2) to getting a firm medium on which fungi could be studied conveniently. Two methods were tried: (1) a large percentage of gelatin was added to the medium, and (2) the medium was made strongly acid by the use of oxalic or lactic acid. The latter method was abandoned, proving largely a failure on account of the tendency toward liquefaction following sterilization. The first method was successful, and was therefore employed for the first platings throughout the investigation. The only difficulty in its use was a tendency with the mineral salts used to give a precipitate, probably magnesium sulphate, during the sterilization. Less of this difficulty occurred by the use of the steam sterilizer than with the autoclave. By the former method a fairly clear medium was obtained, on which practically no bacterial growth appeared.

For the pure culture tubes, an agar medium without gelatin was found most satisfactory, on account of the easy liquefaction of the gelatin in warm weather. Again, a medium containing calcium nitrate instead of ammonium nitrate was found to produce more characteristic cultures of some fungi, since with the latter a cheesy consistency was developed on the medium, and a slimy crust was often formed over the surface, which interfered with the vegetative development. After considerable experimentation, three media were finally used: medium 1 was used almost exclusively for the first platings; the growth of the fungi was also tested on this medium in pure cultures; media 2 and 3 were used ex-

clusively for the pure cultures. These media had the following composition:

## MEDIUM 1

Distilled water.....	100.0 cc.
Gelatin.....	30.0 gm.
Agar.....	2.0 gm.
Monopotassium phosphate.....	0.2 gm.
Ammonium nitrate.....	0.2 gm.
Magnesium sulphate.....	0.02 gm.
Sodium chloride.....	Trace

## MEDIUM 2

The same as medium 1, except the gelatin was omitted.

## MEDIUM 3

Water.....	100.00 cc.
Agar.....	2.00 gm.
Monopotassium phosphate..... (M/100)	0.136 gm.
Calcium nitrate..... (M/100)	0.164 gm.
Magnesium sulphate..... (M/1000)	0.012 gm.
Cane sugar..... (M/100)	0.270 gm.

Medium 3 was not quite so transparent as medium 2. However, as previously noted, the fungi studied seemed to develop more characteristically on the latter. The acidity of media 1 and 2 was tested by the method in common use among bacteriologists (14), described by DUGGAR (32). Medium 1 gave an acidity of 80, according to FULLER'S (34) scale; medium 2 gave 20. The inhibiting effect of the gelatin medium on bacteria is thought to be due to its rather strong acidity. The quantities of the mineral constituents of these media were based on some studies on "optimum media" carried on in this laboratory, where it has been found that the optimum quantities, especially of the nitrate, are much below the quantities generally recommended. The same has been confirmed in this investigation.

## METHOD OF SAMPLES, PLATINGS, AND ISOLATIONS

The method of taking the soil samples was a slight modification of that used by KING and DORYLAND (35), at the Kansas Experiment Station, in bacteriological work. The sampler consists of a steel tube, 10 cm. long and 1 cm. inside diameter, together with a

brass rod, 11 cm. long and 1 cm. in diameter, made to operate as a piston inside the tube. At one end of the tube is a collar through which operates a small thumbscrew for holding the piston in any position desired. The opposite end is sharpened for entering the soil. The brass rod is graduated so as to indicate the size of each soil sample in cc. At the thumbscrew end, the brass rod is rounded to form a convenient knob for holding. For use with the sampler, a lath 20 cm. long and 5 cm. wide was perforated with a row of holes, each slightly larger than the sampler, running lengthwise of the lath and 2 cm. apart from center to center.

The samples were taken as follows: first a hole, about 30 cm. square and 20 cm. deep, was dug with a spade in the experimental plat. Then a vertical face of this hole was scraped off with a sterilized steel spatula, and the fresh surface immediately covered by the lath. Next, the sampler, having been set for the size of sample desired, and sterilized in an alcohol flame immediately before use, was pushed horizontally into the soil through one of the holes in the lath at the desired depth. After being turned once or twice on its long axis to loosen the sample, the sampler was removed and the sample was pushed quickly into a sterilized, cotton-stoppered test tube provided for the purpose. The cotton plug was held between the fingers while putting in the sample, and was returned as quickly as possible. By this method, all surface contamination was avoided and only fungi actually existing in the soil at the depth of the sample were taken. A view of the whole sampling outfit is given in fig. 1.

Samples of 2 cc. each were taken, and after being transferred to the sterile, cotton-stoppered test tubes, were carried to the laboratory and each treated with 18 cc. of sterile distilled water. Each sample was thoroughly shaken, and as soon as the coarser soil particles had settled, 2 or 3 drops were transferred, by means of sterilized pipettes, to the first of a series of three tubes, each of which contained 10 cc. of the gelatin medium, which had been melted previously and maintained at 42°-46° C. From the first of these three tubes 2 or 3 drops were transferred to the second, and from the second 2 or 3 drops to the third. Plates were then poured in the usual way.

Vigorous growth was generally obtained in 3 or 4 days, this time being shortened by higher temperatures. As fast as individual mycelia could be distinguished, pure cultures were isolated by transferring to properly sterilized tubes of media. Medium 2 or 3 was usually used for this purpose, on account of the tendency of the gelatin to liquefy in warm weather. Little difficulty was encountered in getting pure cultures in this way. That the method of plating was successful in keeping out all foreign spores was demonstrated by a set of blanks, which was made at the time the other plates were poured. These were poured like the others, but

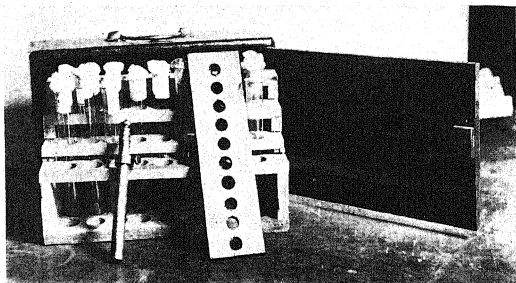


FIG. 1.—Sampling outfit

were inoculated from sterile distilled water. On these, no fungi developed until many days after liberal growth had occurred on the soil-inoculated plates, and then only an occasional mycelium appeared on the very edge of the plate. In most cases, these plates were perfectly clear for weeks after their preparation.

#### DISTRIBUTION AS TO DEPTH

In accordance with a previously stated purpose, these fungi, after isolation, were studied with reference to the following points: (1) distribution as to depth; (2) distribution as to treatment of soil; (3) structural characters and identification. These will be considered in the order given.

The studies of distribution according to depth were made on all three of the plats previously indicated. Samples were taken in different positions horizontally on each plat. Three depths were tried at first: 2 cm., 8 cm., and 14 cm. Later, samples were taken at only two depths: 4 cm. and 12 cm. Four sets of samples were taken on dates and at depths indicated in table I. A series indicates a set consisting of a sample at each level given.

TABLE I

Date	Plat	No. of samples	Depths in cm.
April 28.....	I	2 series	2, 8, 14
July 12.....	I	2	2, 8, 14
12.....	II	2	2, 8, 14
August 15.....	III	1	2, 14
October 12.....	I	1	4, 12
12.....	II	1	4, 12
12.....	III	1	4, 12

After mycelia had become 3-8 cm. wide, countings were made of each plate, as accurately as possible without special device, to determine the number of mycelia for each plate. The counting for each sample included all the mycelia on the three plates which were inoculated from that sample. A summary of the results obtained by these countings is given in table II. The averages given were obtained by adding together all the countings made at a particular depth and then dividing by the number of samples at that depth.

TABLE II

PLAT	AVERAGE NUMBER OF MYCELIA PER SAMPLE AT EACH DEPTH				
	2 cm.	4 cm.	8 cm.	12 cm.	14 cm.
I.....	68.6	21.5	52.3	29.0	54.3
II.....	101.5	32.5	59.5	35.5	50.0
III.....	17.8	36.5	.....	28.0	20.0
Average for 3 plats.....	62.6	30.2	55.9	30.8	41.4

While the accuracy of the absolute values of these countings, from which these averages are computed, might well be questioned, nevertheless, it is believed that the relative values of the averages

are significant. The method of counting was probably not strictly accurate, and, furthermore, the difference in the times of taking samples might well affect the absolute numbers. It is also true that the investigation of a larger number of samples would be desirable before drawing final conclusions. Nevertheless, the comparative results indicate that the fungi in the soil investigated were distributed rather uniformly at different depths, at least as low as 14 cm. No samples were taken at a greater depth. This result is quite different from that usually found for bacteria (see KING and DORYLAND 35).

Some study was also made of the depths at which particular fungi were found. From the results obtained, no definite relationship was discovered. Any particular species seemed about as abundant at one depth as at another, down as deep as samples were taken. The results for the three most abundant species are given in table III.

TABLE III

NAME	TOTAL NUMBER OF ISOLATIONS	NUMBER OF CULTURES AT EACH DEPTH				
		2 cm.	4 cm.	8 cm.	12 cm.	14 cm.
<i>Fusarium</i> (sp. ?).....	13	2	1	5	3	2
<i>Mucor ambiguus</i> .....	14	4	3	1	3	3
<i>Trichoderma nigro-virens</i> .....	8	3	4	0	0	1

These data were obtained, not from the original plate cultures, but only from the pure cultures isolated from the plates. This was for the reason that the fungi were many of them not identified until after they had been isolated for some time, when those in the plates had overgrown each other too far to be distinguishable. The data here given are regarded as insufficient for the drawing of final conclusions, but as far as they go, they do not seem to show any definite relationship between the position of particular species and depth.

#### DISTRIBUTION ACCORDING TO TREATMENT OF SOIL

As previously stated, the three plats were differently treated as to tillage and fertilization. It was expected that a marked difference would be found in the flora of the different plats, espe-



cially in plat III as compared with the others. This plat was heavily manured, and then well spaded and raked. The results, however, did not seem to fulfil the expectation mentioned. Out of about 60 pure cultures isolated, representing 18 different species, but 2 species were found exclusively on plat III, while 3 were found exclusively on plat I, and 3 on plat II. It would seem more reasonable to suppose that these differences were due rather to the chances of sampling or isolation than to differences in the flora of the plats. The results would indicate then a probability, at least, that there is a rather constant and characteristic fungus flora in the soil, regardless of the treatment as to tillage or manuring. It should be noted in this connection that the samples from the manured plat were not taken until about 3 months after the manure was applied. Any foreign fungi, therefore, which might have been introduced with the manure may have begun to grow, and, finding conditions unfavorable, have died out.

The conclusion that there is a rather constant and distinct fungus flora in the soil is strongly confirmed by the work of other investigators. Mr. GROSSMAN, whose work has not yet been published, has worked in this laboratory on a very different soil from that used in this investigation. The soil used by him was a very fine, red clay on which plants grow poorly, located about a mile away from the University. Out of about 12 or 14 species isolated from this clay soil, at least 8 are the same as those reported here. What seems even more remarkable in this connection is that out of 18 species isolated here, 7 are the same as those found by OUDEMANS and KONING (29) in forest soil near Amsterdam, Holland. And furthermore, 4 of these were named by OUDEMANS as new species. Out of 13 genera found here, 8 were the same as those found in Holland.

#### LIST OF FUNGI ISOLATED

The list of fungi isolated is as follows (p. 262). One asterisk indicates fungi found also by GROSSMAN; two asterisks, those found also by OUDEMANS and KONING (29); three asterisks, those found in all three investigations. The descriptions follow, and drawings may be found in figs. 2-16.

PHYCOMYCETES.—(1) *Mucor ambiguus* Vuill.; (2) *M. stolonifer* Ehrenb.  
HYPHOMYCETES.—(3) \**Myceliophthora sulphurea*, n. sp.; (4) *Coccospora*  
*agricola*, n. sp.; (5) \**Fusarium*, sp.; (6) \*\**Acrostalagmus cinnabarinus*  
Cda.; (7) \**Pachybasium hamatum* (Bonord); (8) \*\**Aspergillus calyp-*  
*tratus* Oudem.; (9) *A. nidulans* (Eidam); (10) \**Penicillium glaucum* Link;  
(11) \**P. bicolor* Fries; (12) \**P. candidum* Link; (13) \*\**P. humicolum*  
Oudem.; (14) \**Hormodendron cladosporioides* Sacc.; (15) \*\**Monilia*  
*Koningi* Oudem.; (16) \*\*\**Stysanus stemonites* (Pers.); (17) *Trichoderma*  
*nigro-virens*, n. sp.; (18) \*\**T. Koningi* Oudem.; (19) *Verticillium chlamy-*  
*dosporium*, n. sp.

## DESCRIPTIONS OF FUNGI

For the identifications the systematic works of ENGLER and PRANTL (36), RABENHORST (37), and SACCARDO (38) were used. The work of OUDEMANS and KONING (29) was also found very useful, and the work of LÉGER (40) was referred to.

*MUCOR AMBIGUUS* Vuill.—*Mycelium* floccose, spreading rapidly, soon covering the substratum and sending aerial hyphae 1-3 cm. above; becomes dusty gray by formation of sporangia. *Hyphae* branched, hyaline, 6-10  $\mu$  in diameter. *Sporangiophores* very variable in length; branching racemose-sympodial, richly mixed. Each method occurs separately and the two are often combined in the same fructification. In the racemose type the terminal sporangia are often larger than the lateral, the whole appearing clustered. In the sympodial branching a cross wall cuts off a sporangiophore, and then a lateral branch forms basal to this wall. The lateral branch continues growth, repeating the process, thus developing a sympodial system. The two types of branching are often combined by one or more branches of a racemose cluster becoming elongated and developing in the sympodial way. Branches of the sympodium 10-100  $\mu$  long. *Sporangia* globular, 60-100  $\mu$  in diameter, larger terminal sporangia 125  $\mu$ , dusty brown in color. *Columella* present, conical to globular, 35-50  $\times$  40-60  $\mu$  in diameter, slowly deliquescent leaving a slight collar. *Spores* elliptical, seldom globular, 3-5  $\times$  5-7  $\mu$  in diameter, smooth.—Fig. 2.

This fungus showed extreme variation in the branching of its sporangiophores. While its diagnosis, so far as branching is concerned, comes very near to *M. globosus* Rabenh., its spores are typically elliptical and not globular. At the same time, the columellas are conical to globular and not pyriform.

These characters correspond much more closely to *M. ambiguus*. Considering all its characters, it was decided to refer it to that species.

This form was found very abundantly in all the cultures made. Its abundance was exceeded only by that of *Fusarium* sp.

***Myceliophthora sulphurea*, n. sp.**—*Mycelium* at first orbicular, white, loose, and tufted in the center with a radiate border; becomes sulphur yellow and finally zoned with pale yellow or white center, alternate white and yellow ridges, and a white, radiate border; reverse side orange yellow; medium little colored. *Hyphae* branched, septate, hyaline, 3-4  $\mu$  broad, easily falling apart into oidia-like segments of very variable size and form, some rounding up into oval or globular conidia, others remaining as undifferentiated cells. *Conidiophores* scarcely differentiated from the mycelium; sometimes whole hyphae break up into oidia, in other cases rows of conidia, with yeastlike branching, form at the ends of certain branch hyphae. *Conidia* exceedingly variable, from undifferentiated cells to oval globular spores 5-10  $\times$  10-16  $\mu$  in diameter; no chlamydospores.—Fig. 3.

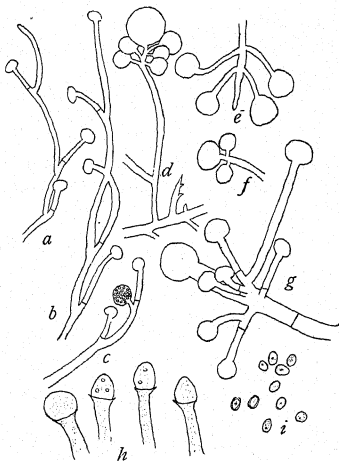


FIG. 2.—*Mucor ambiguus* Vuill.: a-c, sympodial branched sporangiophores,  $\times 80$ ; d-f, racemose sporangiophores,  $\times 80$ ; g, mixed racemose-sympodial sporangiophore,  $\times 180$ ; h, columellas,  $\times 180$ ; i, spores,  $\times 380$ .

The vegetative appearance of this form is very characteristic and very constant, but the spores and method of fructification are rudimentary and very irregular.

*Coccospora agricola*, n. sp.—*Mycelium* orbicular, at first white with a tufted center and radiate border, becoming slightly zoned with concentric grooves and ridges, turning pinkish brown especially inside, finally forming a powdery pinkish brown surface with age; reverse side weakly orange. *Hyphae* very little branched, septate, 4–6  $\mu$  broad, hyaline. *Conidiophores* little differentiated, consisting

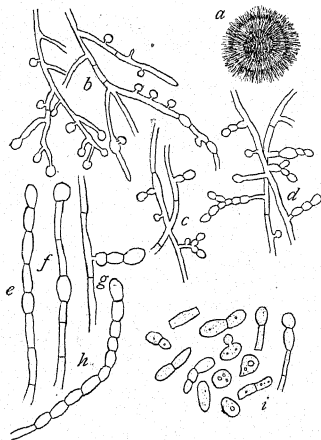


FIG. 3.—*Myceliophthora sulphurea*, n. sp.: a, habit sketch; b, c, e, hyphae bearing simple conidiophores with conidia,  $\times 180$ ; d–h, hyphae breaking up into oidia,  $\times 380$ ; i, oidia-like conidia,  $\times 380$ .

of short side branches, each bearing a single spore at the end, or sometimes forming racemose clusters; side branches 12–30  $\mu$  long, generally septate. *Conidia* (chlamydospores) large, thick-walled, mostly globular, very persistent, not being set free in water, 16–25  $\mu$  in diameter; membrane hyaline, 2–3  $\mu$  thick; contents highly granular and faintly brownish.—Fig. 4.

The large, thick-walled, persistent chlamydospores, and the simple method of fructification, were the most strikingly characteristic features of this fungus.

*FUSARIUM* (sp. ?).—

*Mycelium* white, changing to pink, spreading rapidly to form a compact, floccose mat of indefinite extent, soon covering the medium; no zonation, reverse color pink, medium colored pink. *Hyphae* profusely branched, septate, rising to form a loose, cottony web above the medium, granular, hyaline, 2.5–3.5  $\mu$  broad, under unfavorable conditions forming knotted chains of chlamydospores. *Conidiophores* at first short side branches bearing a spore at the end, or lacking with the

spore sessile; later developing into a *Cephalosporium* stage in which the conidiophore lengthens, becoming 30–80  $\mu$  long, cutting off successive conidia (microconidia) at the end, older conidia pushed aside by the younger to form a globular head enveloped in more or less slime; head falling to pieces easily. *Conidia* varying widely in size, form, and number of septa. *Microconidia* small and kidney or oval-shaped, with no septa, 3–4  $\times$  8–12  $\mu$  in diameter. *Macroconidia* typically sickle-shaped, with 1–4 septa when old; 2-septate spores 4  $\times$  11–20  $\mu$ ; 3-septate spores 4–5  $\times$  25–36  $\mu$ ; 4-septate spores 5  $\times$  38  $\mu$ . *Conidial heads* globular, 30–35  $\mu$  in diameter.—Fig. 5.

This was the most abundant fungus found. It was rather uniformly distributed in all the plats and at all the depths. Ten or twelve mycelia of this were obtained on the plates to one of any other fungus.

ACROSTALAGMUS CINNABARINUS Cda.—RABENHORST 37, 12: fig. 340; OUDEMANS and KONING 29, pl. 6.—*Mycelium* orbicular, at first white, changing to orange or rose pink, becoming zoned with white margin and zones of light and dark pink or orange within; reverse color orange or pink; medium not colored. *Hyphae* branched, septate, 3–4  $\mu$  broad. *Conidiophores* upright, septate, twice verticillately branched, secondary branches 12  $\mu$  long, ninepin form, bearing at the end a globular head of conidia

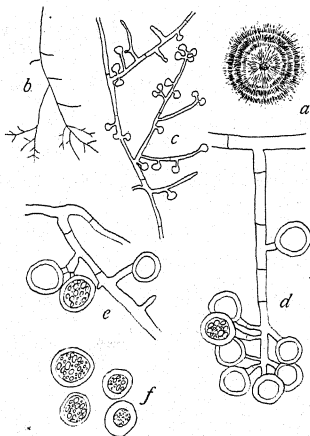


FIG. 4.—*Coccospora agricola*, n. sp.: a, habit sketch; b, branched hypha,  $\times 80$ ; c, hyphae bearing conidia on simple side branches,  $\times 180$ ; d, clustered conidiophores,  $\times 380$ ; e, simple conidiophores bearing conidia,  $\times 380$ ; f, conidia (chlamydospores),  $\times 380$ .

enveloped in slime; heads 8-14  $\mu$  in diameter, easily falling apart. *Conidia* elliptical, 1.5-3 $\times$ 4-6  $\mu$  in diameter.—Fig. 6, A.

This fungus, in most of the cultures, did not show the characteristic rose-red color, but rather a decided orange. However, several luxuriant plate

cultures showing brilliant rose pink were developed on medium 1 by inoculation from an orange culture. Orange cultures were obtained again by inoculation from the rose pink.

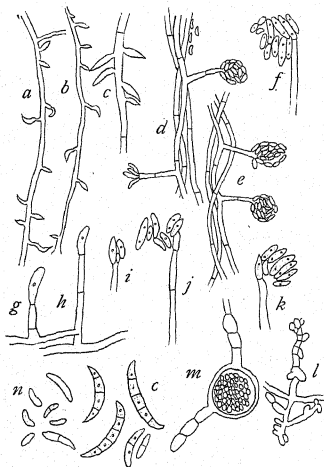


FIG. 5.—*Fusarium* (sp. ?): a, b, hyphae bearing simple conidiophore,  $\times 160$ ; c, same,  $\times 380$ ; d, e, hyphae bearing conidial heads (microconidia),  $\times 180$ ; f-j, stages in formation of conidial heads,  $\times 380$ ; l, hyphae forming chlamydospores,  $\times 180$ ; m, same,  $\times 380$ ; n, microconidia,  $\times 380$ ; o, macroconidia,  $\times 380$ .

**PACHYBASIIUM HAMATUM** (Bonord).—RABENHORST 37, 1<sup>2</sup>: fig. 311; ENGLER and PRANTL 36, 1<sup>1</sup>: fig. 440, A.—*Mycelium* at first white and scant, spreading to form a loose web, sometimes hardly visible on the substratum, soon forming irregular, white, woolly, hemispherical tufts 1-5 mm. broad, giving a scattered, botryoidal appearance; tufts at first white, becoming gray-green as spores form; reverse color the same; no coloration of the medium. *Hyphae* sparingly branched, hyaline, 3-5  $\mu$  broad. *Conidiophores*

rising upright from the hemispherical tufts, consisting of a twice or three times branched fertile portion and an elongated, terminal, sterile portion; fertile portion verticillately branched or forked to branches of the third order; end branches thick flask-shaped, 5-8  $\mu$  long, each having a sterigma-like end which bears a spore; sterile

ends with one or two short branches, septate, bent, and rising over the surface of the tufts giving a characteristic woolly appearance. *Conidia* single, pale green, gray-green in mass,  $3-4 \times 5-7 \mu$  in diameter.—Fig. 7.

This description agrees well with RABENHORST, except that the sizes of the spores and of the flasklike end branches of the conidiophores are a little small. However, there seems little doubt that this is the same species.

**ASPERGILLUS CALYPTRATUS** Oudem.—OUDEMANS and KONING 29, pl. 13.—*Mycelium* floccose, spreading into a thick, white mat of irregular extent, little zonation, becomes avellaneous with age, green where spores are produced thickly changing to almost black; reverse side avellaneous to fulvous; no coloration of the medium. *Hyphae* richly branched, hyaline, septate,  $3-4 \mu$  broad. *Conidiophores* upright or inclined, 0.3–0.4 mm. high, base hyaline and septate, swollen end elliptical or reverse pear-form. *Conidial fructification* long, cylindrical, dark green changing to black,  $40-60 \times 100-200 \mu$  long. *Basidia* nearly cylindrical, pointed, densely packed,  $6-8 \mu$  long. *Conidia* in long chains, globular, smooth, gray-green,  $2-3 \mu$  in diameter.—Fig. 8, A.

**ASPERGILLUS NIDULANS** (Eidam).—ENGLER and PRANTL 36, t: fig. 215.—*Mycelium* at first white, soon becoming chrome green with white or cream border, finally ochraceous to dirty green;

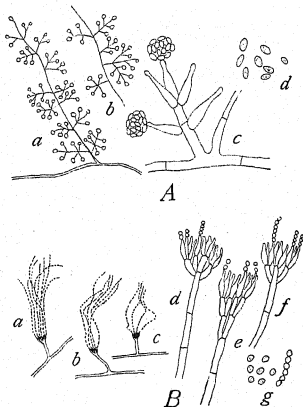


FIG. 6.—A, *Acrostalagus cinnabarinus* Cda.: a, b, hyphae bearing conidiophores,  $\times 80$ ; c, conidiophore with conidiiferous cells and conidial heads,  $\times 380$ ; d, conidia,  $\times 380$ .

B, *Penicillium bicolor* Fries: a-c, conidial fructifications,  $\times 80$ ; d-f, same, showing branching and conidiiferous cells,  $\times 380$ ; g, conidia,  $\times 380$ .

surface having a botryoidal appearance especially on media 1 and 2; reverse color ochraceous; medium colored reddish brown. *Hyphae* much branched, septate, hyaline, 4-5  $\mu$  broad. *Conidiophores* upright, 0.1-0.3 mm. long, septate; swollen end subglobular, 16-23  $\mu$  in diameter. *Conidial fructification* irregularly

globular, loose, dark green to black, 75-125  $\mu$  in diameter. Basidia once branched, primary branches club-shaped, about 5  $\mu$  long; secondary branches ninepin form, 7  $\mu$  long. *Conidia* in long chains, globular, 2.5-3  $\mu$  in diameter.—Fig. 8, B.

**PENICILLIUM BICOLOR** Fries.—*Mycelium* at first white, center becomes glaucous with white border, later develops a sulphur yellow outer zone which continues to form in the new growth, giving slight zonation, the whole finally changes through avellaneous to brown when old; yellow develops more brilliantly on ammonium nitrate media (nos. 1 and 2); less yellow and more glaucous on calcium nitrate medium (no. 3); reverse color fulvous to ferruginous; medium colored brown. *Hyphae*

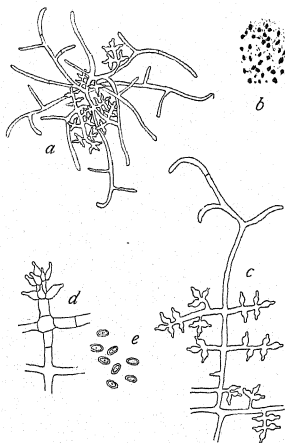


FIG. 7.—*Pachybasium hamatum* (Bonord): a, habit sketch; b, fruiting tuft showing conidiophores and sterile ends,  $\times 80$ ; d, branching conidiophore with whorls of conidiiferous cells,  $\times 380$ ; c, fruiting hyphae with sterile end,  $\times 180$ ; e, conidia,  $\times 380$ .

branched, many septa, much curved, 1.2-2  $\mu$  broad. *Conidiophores* upright, septate, hyaline, 200-300  $\mu$  long, 2-3  $\mu$  broad, branched 1-3 times at the top to form a compact fructification. *Conidial fructification* 100-200  $\mu$  long, consisting of branches to the second and at times to the third order (tertiary), the last bearing whorls of



conidiiferous cells on the end of which are long chains of conidia; fructification very compact; branches cylindrical, of about equal length, 8–10  $\mu$  long, 3  $\mu$  broad. *Conidiiferous cells* slender flask-form, slightly bent, 8–9  $\mu$  long. *Conidia* globular, nearly hyaline, pale green in mass, 2–3  $\mu$  in diameter.—Fig. 6, B.

This answers closely to the fungus described by OUDEMANS and quoted in RABENHORST under the name *bicolor*, as found in humous earth in Holland. I find nothing in THOM (33) that seems to correspond to it. It would appear to me that this form has decided specific characters, which distinguish it sharply from either *P. glaucum* Link or *P. crustaceum* L.

**PENICILLIUM CANDIDUM** Link.—*Mycelium* spreading, floccose, pure white slowly changing to glaucous, sometimes remaining perfectly white especially on media 1 and 2, finally gray to avellaneous in the oldest parts; reverse color yellowish to ochraceous or even ferruginous; medium uncolored. *Hyphae* branched, septate, about 3  $\mu$  broad. *Conidiophores* up to

250  $\mu$  long, 3–4  $\mu$  broad, septate, hyaline, branched once or twice, often not at all, 1–5 conidiiferous cells at the end. *Conidiiferous cells* ninepin form, 9–12  $\mu$  long, 3–4  $\mu$  broad. *Conidial fructification* 100–150  $\mu$  long, often longer, loosely brushlike. *Conidia* smooth,

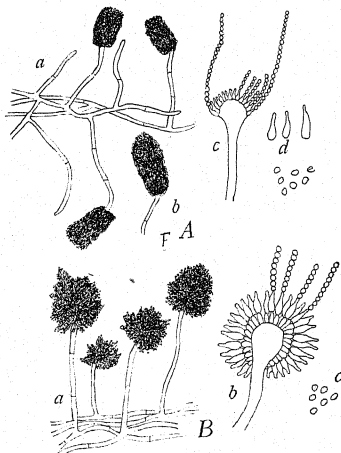


FIG. 8.—A, *Aspergillus calypttratus* Oudem.: a, b, conidiophores with conidial fructifications,  $\times 80$ ; c, conidial head with sterigmata and conidial chains,  $\times 380$ ; d, sterigmata,  $\times 750$ ; e, conidia,  $\times 380$ .

B, *Aspergillus nidulans* (Eidam): a, conidiophores with conidial fructifications,  $\times 80$ ; b, conidial head with sterigmata and conidial chains,  $\times 380$ ; c, conidia,  $\times 380$ .

thin-walled, pale blue-green by transmitted light,  $2 \times 3 \mu$  in diameter, varying to globose.—Fig. 9.

This description does not fit *P. candidum* perfectly. The spores of the form here described are oval and pale blue-green, while those of *P. candidum* are described as globular and white. However, these do vary to globular, although not typically. Again, the changing of the mycelium from white to gray-green and avellaneous is unlike *P. candidum*. However, this character varied on different media. On no. 3, the gray-green showed more decidedly than on 1 or 2. The white followed by avellaneous was more decided on no. 2. The color characters and also the spores seem to correspond more closely to the *P. camemberti* described by THOM. Differences in the other characters, however, would not at all favor this name. On the whole it has seemed best to call it *P. candidum*.

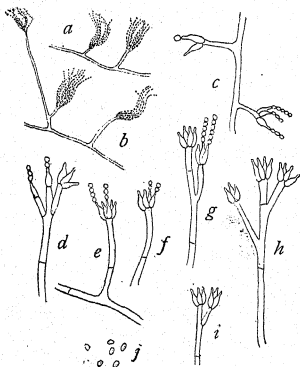


FIG. 9.—*Penicillium candidum* Link: a, b, conidial fructifications,  $\times 80$ ; c-i, conidial fructifications with conidiiferous cells,  $\times 380$ ; j, conidia,  $\times 380$ .

**PENICILLIUM HUMICOLUM**  
Oudem.—OUDEMANS and  
KONING 29, pl. 26.—This  
fungus showed marked differ-  
ences when grown on different  
media. On media 1 and 2,  
the *mycelium* was cream to  
yellowish green, forming a  
wrinkled, cheesy, superficial  
crust which becomes dark  
green (*atro-virens*) over por-

tions where spores form; mat of limited extent, usually orbicular; no zonation, but with a narrow cream colored border; reverse color yellow (*melleus*); no coloration of the medium. When grown on medium 3, the *mycelium* formed no yellowish, wrinkled crust, but produced an orbicular, dark green mat, somewhat raised in the center and having a narrow white border; reverse color yellowish. *Hyphae* richly and irregularly branched, very irregular in form, showing constrictions and enlargements giving a gnarled appearance, many septa, short cells, contents foamy and vacuolated.

*Conidiophores* erect, little branched, hyaline, septate, up to  $130\ \mu$  long, often very short,  $3\text{--}4\ \mu$  broad. *Conidial fructification* long, narrow, compact,  $75\text{--}120\ \mu$  long,  $15\text{--}20\ \mu$  broad, branched trichotomously; primary branches tapering, bent,  $8\text{--}10\ \mu$  long; secondary branches usually 3, at times 4, flask-form,  $5\text{--}7\ \mu$  long. *Conidia* in long chains, hyaline, globular,  $2\text{--}3\ \mu$  in diameter.—Fig. 10.

This comes very close to OUDEMANS' *P. humicolum*, although the breadth of the conidiophores and of the spores is slightly larger for the fungus here described. However, there was considerable variation in these sizes.

HORMODENDRON CLADOSPORIOIDES Sacc.—SORAUER 41, fig. 7.—

*Mycelium* orbicular, smoky changing to black, slightly zoned with fringelike border; surface of mat becomes botryoidal and much wrinkled; reverse color black; medium not colored. *Hyphae* much branched, septate, somewhat gnarled, smoky to brown (umbrinus),  $3\text{--}5\ \mu$  broad. *Conidiophores* a little branched at the top, septate,  $75\text{--}100\ \mu$  long,  $5\text{--}8\ \mu$  broad. *Conidial fructification* a dense, irregular mass of spores borne in chains with yeastlike branching. *Conidia* in branched chains, ends blunt or pointed, oval to elongate,  $3\text{--}5 \times 6\text{--}15\ \mu$  in diameter.—Fig. 11.

MONILIA KONINGI Oudem.—OUDEMANS and KONING 29, pl. 21.—*Mycelium* white and finely floccose, forming a light brown

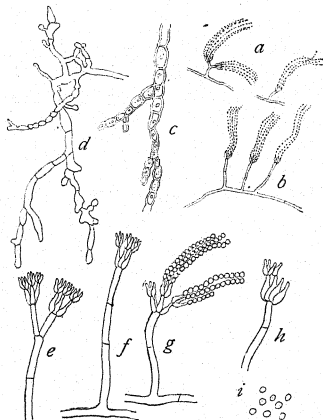


FIG. 10.—*Penicillium humicolum* Oudem.: a, b, conidial fructifications,  $\times 80$ ; c, hyphae showing foamy, vacuolated condition,  $\times 380$ ; d, gnarled hyphae; e-h, conidial fructification showing conidiiferous cells and conidial chains,  $\times 380$ ; i, conidia,  $\times 380$ .

(light umbrinus) center which spreads and becomes somewhat zonate by varying densities of color, the whole finally becomes powdery brown; reverse color brown to ferruginous; no coloration of the medium. *Hyphae* branched, septate, hyaline, 4-5  $\mu$  broad. *Conidiophores* little differentiated from the mycelium, septate, once

or twice branched, the last branching often being di- or trichotomous, each division bearing a long conidiiferous cell. *Conidiiferous cells* flask-form, 30-40  $\mu$  long, often arising singly on simple side branches. *Conidial fructification* either simple or clustered, each flask-shaped cell bearing a long chain of conidia. *Conidia* in chains up to 30, often connected by a neck, terminal conidia often larger, globular to lemon-form, 6-9  $\mu$  in diameter, brown (light umbrinus), chains persistent.

—Fig. 12.

On the ammonium nitrate media (nos. 1 and 2), this fungus developed differently in its general appearance. The mycelium often formed a cream colored, cheesy, irregular crust with a loose network of hyphae

about the edges. This crust was finely zonate and had radiate grooves about the border. Later, spores formed thickly over the surface of the crust, giving a powdery, brown surface. Abundance of moisture favored this cheesy formation.

STYSANUS STEMONITES (Pers.).—RABENHORST 37, t<sup>2</sup>: fig. 337; OUDEMANS and KONING 29, pl. 29.—*Mycelium* at first white,

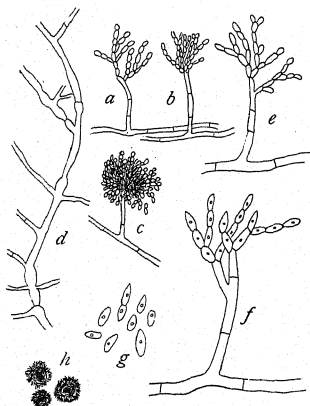


FIG. 11.—*Hormodendron cladosporioides* Sacc.: a, b, hyphae with conidiophores and conidial fructifications,  $\times 180$ ; c, compact conidial fructification,  $\times 180$ ; d, branching hyphae,  $\times$  about 300; e, conidiophore and fructification,  $\times$  about 300; f, same,  $\times 380$ ; g, conidia,  $\times 380$ ; h, habit sketch.

radiate, hemispherical, soon dark brown (dark umber) in the center, finally dusty brown with tufts of coremia, the whole having a narrow white border; reverse color rusty to dark brown; medium often changed to creamy, cheesy mass especially on ammonium nitrate media (nos. 1 and 2). *Hyphae* branched, hyaline, septate,  $3.3-4.1\ \mu$  broad. *Coremia* in bushlike tufts 1-5 mm. in diameter; single coremia 1-3 mm. high, made up of a slender stalk and an expanded, cylindrical, brush-like head; head made up of long hyphae which send out short side branches each of which bears a single basidium or branches trichotomously, each division bearing a basidium-like cell. *Basidia* flask-shaped,  $5-7\ \mu$  long, each bearing a long chain of spores. Spores are also produced in simple, umbellate clusters which are borne on short side branches. These clusters form especially at the edge of the medium. *Conidia* in long chains, oval to lemon-shaped, sometimes joined by a short neck,  $3.5-4.5 \times 6-8\ \mu$  in diameter, pale bluish green when single, umber brown in mass.—Fig. 13.

*Trichoderma nigro-virens*, n. sp.—*Mycelium* orbicular, at first white, spreading to form a thick, floccose mat, later developing successive concentric, dark green (nigro-virens) zones alternating with white, green zones preceded by white granular zonation, dark

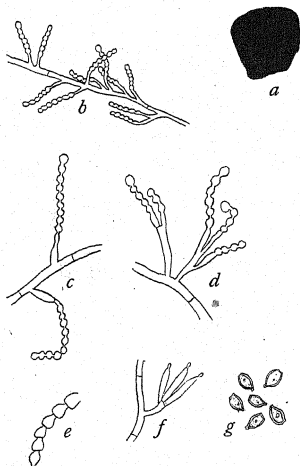


FIG. 12.—*Monilia Koningi* Oudem.: a, habit sketch; b, conidial fructification,  $\times 80$ ; c, d, same,  $\times 160$ ; e, chain of conidia,  $\times 380$ ; f, conidiiferous cells,  $\times 380$ ; g, mature spores,  $\times 380$ .

zones finally almost black, sometimes brown to olive green, giving a very characteristic and prominent black and white zonation with a broad white border; reverse color sulphur yellow to reddish brown on agar-ammonium nitrate medium; medium colored yellow. *Hyphae* richly branched, hyaline, septate,  $1.7-2.4\ \mu$  broad.

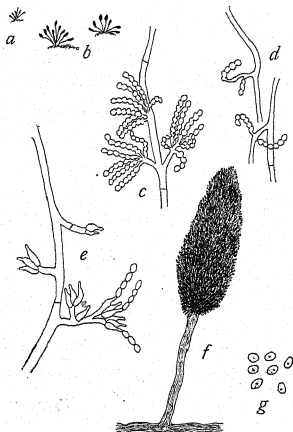


FIG. 13.—*Styxanus stemonites* (Pers.): a, tuft of coremia, nat. size; b, tufts of coremia,  $\times 2.5$ ; c, d, conidial fructifications,  $\times 180$ ; e, conidiophores with conidiiferous cells and chains of conidia,  $\times 380$ ; f, coremium,  $\times 80$ ; g, conidia,  $\times 380$ .

*Conidiophores* branched, branches opposite or alternate, two or three times forked at the end, each end branch bearing 2 or 3 conidiiferous cells, each of which bears a conidial head at the tip. *Conidiiferous cells* long, tapering, abruptly pointed,  $15-20\ \mu$  long,  $3-4\ \mu$  broad. *Conidial heads* irregularly globular,  $4-8\ \mu$  in diameter when young, massed together with age to form black, granular, masses  $100-500\ \mu$  in diameter which form the black zones; conidiophores at last disappear, leaving only a mass of spores, whose arrangement often simulates chains. *Conidia* oval to fusiform, green by transmitted light, dark green to

black in mass,  $3-5 \times 6-8\ \mu$  in diameter.—Fig. 14.

This was the third most abundant fungus found. It occurred in nearly all the plates. Its identification was especially difficult because of the rapidity with which the conidial heads massed together, making it hard to determine the character of the conidiophores. This was especially confusing on account of the chainlike arrangement of the spores in the granular masses. The black and white zonation was the most conspicuous character in the manner of growth. In some cultures, the spore masses fused together in the presence

of abundance of moisture to form a continuous, inky black, somewhat slimy surface.

**TRICHODERMA KONINGI** Oudem.—**OUDEMANS** and **KONING** 29, *pl. 31*.—*Mycelium* sparse, loose, spreading indefinitely, at first white and floccose, later bluish green and finally dark green (*atro-virens*) or olive green, spreading loosely and irregularly over the surface; reverse color olive green; no coloration of the medium. *Hyphae* richly branched, scattered, hyaline, septate,  $2.5\text{--}3.5\ \mu$  broad, forming a loose web scarcely covering the medium. *Conidiophores* branched, opposite or alternate, twice or three times forked, each branch ending in 1–3 conidiiferous cells each of which bears a conidial head. *Conidiiferous cells* flask-shaped,  $8\text{--}10\ \mu$  long,  $3\text{--}4\ \mu$  broad. *Conidial heads* globular, without slime,  $6\text{--}10\ \mu$  in diameter, easily breaking to pieces. *Conidia* pale green, transparent, elliptical varying to globular,  $2.5\text{--}3 \times 3\text{--}4\ \mu$  in diameter.—Fig. 15.

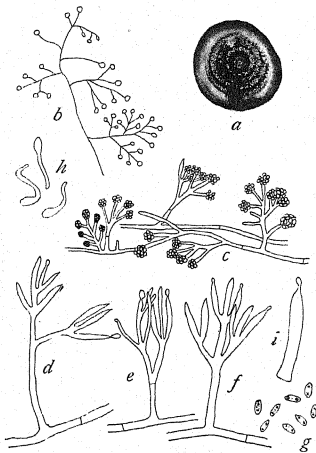


FIG. 14.—*Trichoderma nigro-virens*, n. sp.: a, habit photograph; b, conidiophores,  $\times 80$ ; c, conidiophores showing conidial heads,  $\times 180$ ; d-f, conidiophores showing conidiiferous cells,  $\times 380$ ; g, conidiiferous cell,  $\times$  about 750; h, conidia,  $\times 380$ ; h, germinating conidia.

**Verticillium chlamyosporium**, n. sp.—*Mycelium* orbicular, spreading into a thick mat with little zonation, at first white, later cream, and finally ochroleucous to ochraceous forming a firm crust; surface powdery with age; reverse color yellow (*flavus*) to orange; no coloration of the medium. *Hyphae* branched, septate, hyaline,  $2\text{--}3.3\ \mu$  broad. *Conidiophores* upright, branched verticil-

lately; end branches  $15-30\ \mu$  long, in whorls of 3 or 4, tapering to a knobbed end which bears a single spore. *Conidia* oval, hyaline,  $2.2-3.5\ \mu$  in diameter, easily falling away. *Chlamydospore* formation especially common; chlamydospores multicellular, 4-9-celled, with granular contents, thick-walled, globular,  $10-25\ \mu$  in diameter when mature, slightly lobed, borne on short side branches which are  $15-30\ \mu$  long, very persistent.—Fig. 16.

This chlamydospore-formation is the most characteristic feature of this fungus. The older mycelium becomes a complete mass of these, matted in with hyphae, which at last disappear, leaving the chlamydospores as a creamy or ochraceous powder. The verticillate, conidial fructification forms more abundantly in the young mycelium at the edges of the culture, while later only chlamydospores are formed.

### III. Assimilation of free nitrogen

#### GENERAL PLAN AND METHOD

In this part of the investigation an effort was first made to get some qualitative indication of nitrogen-fixation in a trial with all the fungi which had been isolated from the experimental plat. After this, it was planned to carry on further investigation with exact analytical methods, using any forms which gave any signs of such power. In carrying out this plan five separate investigations were made.

1. Culture solutions of 25 cc. each were prepared in large test tubes, which, after sterilization and inoculation by means of spores, were allowed to stand in the air protected only by germ-proof cotton stoppers.

2. A similar investigation was carried out, using 150 cc. Erlenmeyer flasks instead of test tubes, and using 50 cc. of the nutrient solution for each culture.

3. Cultures of four fungi, which gave the most hopeful indication of nitrogen, were prepared in an apparatus for aerating by means of air drawn through sodium hydroxide and concentrated sulphuric acid. Each culture contained 100 cc. of the culture solution.

4. Cultures similar to those in "3" were put under bell jars



which allowed entrance of air only through U-tubes containing concentrated sulphuric acid.

5. Cultures of one of these four fungi (*Myceliophthora sulphurea*) were made in Erlenmeyer flasks containing solutions with varying quantities of ammonium nitrate, to see whether nitrogen-fixation would not be possible when growth was started by small quantities of combined nitrogen. These cultures, each containing 50 cc. of the nutrient solution, were left exposed to the laboratory air, being closed only by cotton stoppers.

The media for all these cultures were made up by the use of ammonia-free water, as shown by tests with Nessler's reagent. This water, for some cultures, was prepared by a second distillation of distilled water, to which had been added a small quantity of sulphuric acid. The first part of the distillate was always discarded as an extra precaution. Some of the cultures were made with extra pure, so-called "conductivity water" obtained from the chemical laboratory of the University of Michigan.

The chemicals used in all the investigations were strictly C.P. Most of them were marked "Kahlbaum." Furthermore, they were all submitted to chemical analysis, which showed all to be nitrogen-free except the dextrose, which contained 0.25 mg. of nitrogen

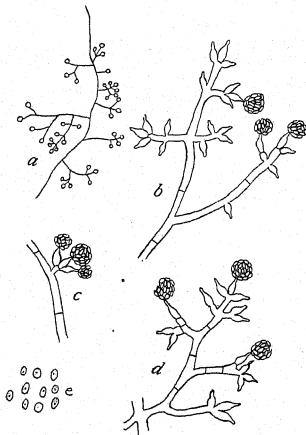


FIG. 15.—*Trichoderma Koningi* Oudem.: a, hyphae with conidiophores,  $\times 80$ ; b-d, conidiophores with conidiiferous cells and conidial heads,  $\times 380$ ; e, conidia,  $\times 380$ .

per 1.0 gm. of dextrose. As pointed out later, this nitrogen did not appear to be in a form which was available to the fungi.

In every investigation, all the glassware for the cultures was cleaned with the greatest care by being placed for 6-24 hours in a mixture of potassium dichromate and concentrated sulphuric acid.

All rubber for connections was new and was first boiled in a 10 per cent solution of sodium hydroxide until perfectly clean.

Sterilizations were carried out in an autoclave raised to 150° C. at least 20 minutes. It was found that temperatures much above this were likely to cause some decomposition of the dextrose, giving a brown coloration of the medium (DUGGAR 32, p. 20).

Inoculations were made in all cases by introducing, with a sterilized platinum loop, a small quantity of spores from a pure culture into about 10 cc. of sterilized distilled water in a small test tube. Then from this one or two drops were transferred to the sterilized culture flasks by means of sterilized pipettes

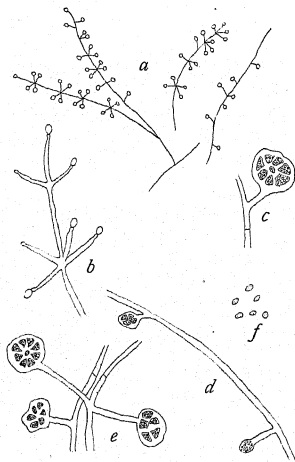


FIG. 16.—*Verticillium chlamydosporium*, n. sp.: a, conidiophores,  $\times 80$ ; b, portion of conidiophore with conidiiferous cells,  $\times 380$ ; c, e, chlamydospores,  $\times 380$ ; d, young chlamydospores,  $\times 380$ ; f, conidia,  $\times 380$ .

held in a corked and germ-protected beaker. That the cultures were inoculated with viable spores was shown by controls, in which spores from the same source were inoculated into nitrogen-containing media. Such controls all showed vigorous and characteristic growth, with the exception of the first investigation as explained hereafter. Furthermore, some growth took place in all

the nitrogen-free cultures, and microscopic examination established the purity of these growths.

The fungi tested in this series of investigations include all those previously given in the complete list, excepting *Mucor stolonifer*, *Penicillium glaucum*, *P. bicolor*, and *Trichoderma Koningi*.

In the last four investigations, cultures were submitted to the most careful chemical analyses to determine the quantity of nitrogen in the culture at the beginning and at the end of the growth period. Proper controls were used in each case.

In all the five investigations above mentioned, the results were entirely negative for all the 15 fungi tried, under the conditions of the investigations. The highest amount of nitrogen found in any culture in a nitrogen-free medium, after the method of analysis had been mastered, was 0.46 mg. in a 50 cc. culture. A repetition of this culture under essentially the same conditions showed only 0.11 mg. gain. One other result of 0.31 mg. gain was obtained, although the duplicate culture showed a gain of but 0.03 mg. Aside from these results mentioned, all the values for the nitrogen gain are well within the limits of error of the method. This error is somewhere about 0.14 or 0.21 mg. Indeed, it is doubtful if results can always be obtained as accurately as this. In the cultures of investigation V, containing ammonium nitrate, the highest nitrogen gain was 0.27 mg. in a 50 cc. culture which contained at the beginning 0.22 mg. fixed nitrogen.

#### CHEMICAL ANALYSES

For the chemical analyses, different modifications of the Kjeldahl method were employed. In general, these methods were in accordance with the official methods of the Association of Official Agricultural Chemists of the United States. For nitrate-free media, the Gunning method was used, modified by the use of copper sulphate, according to the investigations by HIBBARD (43) and in accord with the method used by TERNETZ (19; see HOPPE-SEYLER 44). For the analysis of media containing ammonium nitrate, the "Official Gunning modification to include the nitrogen of nitrates" was used, modified by the use of copper sulphate as in

the "nitrate-free method." The details for these methods were as follows:

1. *For the nitrate-free method.*—The material to be analyzed was first placed in the Kjeldahl flask. This material was either in the form of a solid, or in the case of solutions was evaporated nearly to dryness as hereafter described. Next, 10 gm. of a mixture of potassium sulphate and copper sulphate were added to the flask. The proportion of these which gave the best results was 9.2 parts of  $K_2SO_4$  to 0.8 parts of  $CuSO_4$ . Then 25 cc. of sulphuric acid was added, after which gentle heat was applied. This was increased gradually to strong heat and this was continued for 15–30 minutes after clearing. The contents of the Kjeldahl flask were now rinsed into a 1000 cc. distillation flask and diluted with 240 cc. ammonia-free water. The whole was made alkaline by the addition of 120 cc. of a strong solution of sodium hydroxide (40 gm. pure sticks to 120 cc. ammonia-free water). Two small pieces of stick zinc (granulated zinc caused too much foaming) were now added, and then heat was applied until about 250 cc. of the distillate had passed over into the standard acid. The volume of the standard acid was in most cases 20 cc.

2. *Method in presence of nitrates.*—The material was added to the digestion flask and when this was thoroughly cool, 30 cc. of a mixture of concentrated sulphuric acid and salicylic acid were added. In most cases these two, previously mixed, were added in the proportion of 1 gm. salicylic to 30 cc. sulphuric; but when large quantities of nitrate were present, 1 gm. of salicylic was used to 25 cc. sulphuric. The flask was now allowed to stand 5–10 minutes with frequent shakings, and then 5 gm. sodium thiosulphate were slowly added and well mixed. Heat was then slowly applied for about 5 minutes or until white fumes began to appear, after which the whole was cooled for 5–10 minutes. Next, 10 gm. of a mixture of potassium sulphate and copper sulphate were added; gentle heat was applied till all foaming ceased, and lastly, strong heat was applied till 15–30 minutes beyond clearing. Distillation was carried out as in the nitrate-free method.

The titration method was used throughout for determining the quantity of ammonia in the distillate. For very small quantities

of ammonia, as in nitrogen-free cultures, the distillate was passed into N/20 sulphuric acid, which was then titrated by the use of N/20 sodium hydroxide. In some cases with larger nitrogen content, N/10 or even N/5 solution of the acid was used. Ammonia-free water was employed for all the distillations. The distilled water of the laboratory was found to contain 0.21 mg. of nitrogen in 300 cc. of the water. The ammonia-free water for this was prepared by the evaporation of distilled water to one-half its original volume after the addition of a small piece of sodium hydroxide.

After the titration, the quantity of nitrogen was calculated as follows: 1 cc. N/10 sulphuric acid is equivalent to 1 cc. N/10 ammonia ( $\text{NH}_3$ ); 1 cc. N/10 ammonia contains 1.404 mg. of nitrogen; then the nitrogen present is equal to the number of cc. of N/10 acid used times 1.404 mg.

All chemicals used in the analyses were the purest that could be obtained. The sulphuric acid was marked "Baker and Adamson, standard purity." At the same time, the purity of the chemicals was tested by a blank in which only the chemicals were used. This showed the chemicals used in the nitrate-free method to contain 0.42 mg. of nitrogen, and in the nitrate method 0.56 mg. The amount of the proper blank was subtracted in each case from the total nitrogen of the determination. The filters used in separating the mycelium from each of the cultures were analyzed and found to be nitrogen-free.

The apparatus employed for the digestion consisted of 500 cc. Kjeldahl flasks of the oval form, each provided with a straight bulb tube, which was inserted in the neck of the flask to prevent loss of sulphuric acid. The distillation was carried out in 500 cc. round bottom Jena flasks. These were connected with Kjeldahl bulbs by means of rubber stoppers, and the bulbs led into glass condensers which were placed in an upright position. The distillate was collected in a 500 cc. Erlenmeyer flask. The burettes used for the titrations were the most carefully standardized, with enamel backs and a blue line for accurate readings. All apparatus and utensils were washed with the greatest care after use each time. In addition, they were rinsed at least twice with distilled water, after which they were either inverted or covered.

The standard solutions were standardized in two ways. First, by titration of the alkali against standard succinic acid, which was prepared by dissolving 5.903 gm. of C.P. salt in 1000 gm. of distilled water. Second, these results were confirmed by reference to standard hydrochloric acid whose absolute strength had been determined by precipitation with silver nitrate and weighing of the silver chloride. The sodium hydroxide was found to gain strength by standing in glass, so this was checked every few days against the standard acid.

Methyl red (45) was employed as the indicator for titrating the distillate. This was found to give a much sharper and more accurate end reaction than either cochineal or methyl orange. The whole of the distillate was used for titration. The results were found much less accurate when only part was used and computation made for the whole. In standardizing solutions, phenolphthalein was usually used as indicator, although with the strength of acids employed, the methyl red was found to be equally accurate.

Culture solutions were evaporated over a water bath, down to about 10 cc., before adding them to the digestion flask. Previous to the evaporation, 5 drops of concentrated sulphuric acid were added to fix any free nitrogen present. This precaution applied especially to the cultures left exposed to laboratory air. In the analyses when ammonium nitrate was present, this evaporation was found to be especially necessary, since the heat resulting from the mixture of the acid and water caused a loss of nitrogen in the form of nitric acid or oxides of nitrogen. To avoid this, extra precautions were taken. After the evaporation had been carried to about 10 cc. in the usual way, this quantity was transferred to a Kjeldahl flask with two or three small rinsings, and then the evaporation was carried nearly to dryness in this flask over a water bath. It was also found necessary to have the contents of the flask perfectly cool before adding the mixture of sulphuric and salicylic acids. To accomplish this, the flask was cooled in ice water and then held in ice water while adding the mixture of acids. As a still further precaution, about 0.5 gm. of salicylic acid was added to the material to be analyzed and thoroughly mixed before adding the sulphuric-salicylic mixture. In using the ammonium nitrate

in the form of the dry salt for the test analyses, it was found necessary to have the salt thoroughly powdered before adding the sulphuric-salicylic mixture. In nearly all cases the analyses were carried through in duplicate.

By both methods of analysis used, a series was carried through with some substance of known composition and purity, in order to make sure of accurate results from the method. Asparagin was used for the nitrate-free method, while ammonium nitrate was employed for the method including nitrates. For all small amounts of asparagin, a suitably large quantity of the pure salt was carefully weighed on an analytical balance and then dissolved in a quantity of water measured up in a volumetric flask. For each analysis, the desired quantity of this solution was measured off into an evaporating dish by use of an accurate burette. The solution was then evaporated down to 5-7 cc., and was then poured into the Kjeldahl flask, together with two or three small rinsings. The following tabulation gives the results of these analyses in comparative view:

TABLE IV

Blank determination gave N 0.42 mg.; calculated per cent of N in asparagin, 18.70; solution 1, 80 mg. asparagin dissolved in 200 cc. distilled water; solution 2, 300 mg. asparagin dissolved in 1000 cc. distilled water.

No.	Material used	N obtained less blank mg. <i>a</i>	Calculated amount mg. <i>b</i>	Difference ( <i>b-a</i> ) mg. <i>c</i>	Percentage obtained <i>d</i>	Variation from calc. percentage <i>e</i>
1	100 mg. dry salt.....	17.97	18.70	0.73	17.97	-0.73
2	100 " " ".....	18.11	18.70	0.59	18.11	-0.59
3	100 " " ".....	18.04	18.70	0.66	18.04	-0.66
4	50 " " ".....	8.98	9.35	0.37	17.96	-0.74
5	50 " " ".....	8.98	9.35	0.37	17.96	-0.74
6	50 " " ".....	8.92	9.35	0.43	17.83	-0.87
7	20 " " ".....	3.79	3.74	0.05	18.90	0.20
8	20 " (50 cc. sol. 1) ..	3.65	3.74	0.09	18.25	-0.45
9	15 " (50 cc. sol. 2) ..	2.67	2.80	0.13	17.80	-0.90
10	15 " (duplicate of 9) ..	2.70	2.80	0.10	18.00	-0.70
11	3 " (10 cc. sol. 2)....	0.53	0.56	0.03	.....	.....
12	3 " (duplicate of 11) ..	0.49	0.56	0.07	.....	.....

The similar analyses were not run in duplicate except as indicated. It will be seen that the greatest variation occurs in the case of no. 7, where a relatively small quantity of dry salt was used.

It seems probable that this is accounted for by the inaccuracy of weighing and transferring so small a quantity, an error which becomes relatively very great in so small an amount. If we eliminate this result, it is quite evident from the table that the nitrogen content of asparagin as shown by the analyses is a little lower than the calculated amount. The difference is very small and without much doubt is attributable to slight impurity of the salt. Considering the 50 and 100 mg. analyses only, we see a variation in the different analyses, not made at the same time, of only 0.28 of 1 per cent. This seems a good degree of accuracy for such determinations. Of course, the per cent of error in quantities as small as 3 mg. of the asparagin salt would have little significance. Considering the absolute variation of the analyses, including 20 mg. and below (not including no. 7), we see only 0.1 mg. difference between the maximum and minimum variation from calculated results. This is true even with slightly impure asparagin. These quantities of nitrogen are, it may be observed, larger than those dealt with in the nitrogen-free cultures. It would seem safe to say then that the limit of error of the method was not far above 0.1 mg. in each determination. After considerable experience with determinations under different conditions, it is believed that this limit might run as high as 0.3 mg. in a long series of determinations.

The weighing up of the mycelium in the cultures was done, as were all other quantitative weighings, on an exact Becker analytical balance which weighed accurately to 0.2 mg. The filters were first dried in an oven at 100° C. until they showed a constant weight (about 6 hours). The culture was then filtered and the filter containing the mycelium was dried to a constant weight. Glass-stoppered weighing bottles were used for all this weighing and drying. As previously stated, the filters were found by a special analysis to be nitrogen-free.

Analyses were also made to test the accuracy of the method when nitrates were present. First, an analysis was made of 400 mg. of dry ammonium nitrate. This was the amount contained in the nitrate cultures of the highest concentration. Next, an analysis was made of the original culture solution from which all the cul-



tures of investigation V were made. This solution contained, in addition to the usual culture constituents, 400 mg. of ammonium nitrate. From this solution 50 cc. were accurately measured from a burette and then diluted to 250 cc. in a volumetric flask. Then 50 cc. of this solution was taken for analysis. This contained 80 mg. of ammonium nitrate. The results of these analyses are given in table V.

TABLE V

Blank by nitrate method, 0.56 mg. N; control with other constituents of the medium, but no ammonium nitrate, 0.70 mg. N; calculated percentage of N in ammonium nitrate, 35.04.

No.	Material	N obtained less blank mg. <i>a</i>	Calculated amount mg. <i>b</i>	Difference ( <i>b-a</i> ) mg. <i>c</i>	Percentage N obtained <i>d</i>	Deviation from calculated percentage <i>e</i>
1	400 mg. dry $\text{NH}_4\text{NO}_3$ .....	138.6	140.1	1.5	34.6	0.4
2	400 mg. (duplicate) .....	139.2	140.1	0.9	34.8	0.2
		N obtained less blank and control				
3	80 mg. (50 cc. of solution).....	27.79	28.04	0.25	34.7	0.3
4	80 mg. (duplicate) .....	27.79	28.04	0.25	34.7	0.3

These results seem to show that the method is sufficiently accurate for detecting any appreciable gain in nitrogen in the cultures containing ammonium nitrate.

## INVESTIGATION I

As previously stated, cultures were made in large test tubes, each containing 25 cc. of the culture medium. This medium had the following composition:

Water (ammonia-free) .....	1000.00 cc.
Rock candy (pure), M/20 .....	17.107 gm.
Monopotassium phosphate, M/100 .....	1.361 gm.
Magnesium sulphate, M/500 .....	0.241 gm.
Calcium carbonate, M/1000 .....	0.100 gm.
For controls, ammonium nitrate was added to the above as follows:	
Ammonium nitrate .....	0.360 gm.

The following 11 different species were used for the inoculations:  
*Mucor ambiguus*, *Myceliophthora sulphurea*, *Coccospora agricola*,

*Fusarium* (sp. ?), *Acrostalagmus cinnabarinus*, *Pachybasium hamatum*, *Aspergillus calypttratus*, *Hormodendron cladosporioides*, *Monilia Koningi*, *Stysanus stemonites*, and *Trichoderma nigro-virens*. Four inoculations were made with each fungus, two in N-free cultures and two in ammonium nitrate cultures. This gave duplicates of all cultures. Growth took place in all of these, except those of *Stysanus* and *Hormodendron*. One of the duplicates for each of these failed to show any germination, and evidence was obtained later that the spores were no longer viable. In one or two cases there were doubts about the purity of cultures. On these accounts, it was decided to make another trial of the whole set. This result should be noted; however, that while a visible amount of growth took place in all these cultures, the amount was extremely small in all N-free cultures, and furthermore, it all took place within two or three weeks, after which no growth was to be observed, although observations were continued for over three months.

#### INVESTIGATION II

The second investigation was carried out along the same general lines as the first, but with several modifications. First, Erlenmeyer flasks of about 150 cc. capacity were substituted for the test tubes. In each flask was placed 50 cc. of the culture medium instead of 25 cc. as before. Second, the culture solution was modified, using a higher percentage of phosphate and nearly doubling the amount of sugar, which was supplied this time in the form of dextrose. The full formula was as follows:

Ammonia-free water.....	1000.00 cc.
Dextrose.....	30.00 gm.
Monopotassium phosphate.....	2.00 gm.
Magnesium sulphate.....	0.20 gm.
Calcium carbonate.....	0.10 gm.
Sodium chloride.....	0.02 gm.
For controls:	
Ammonium nitrate.....	2.00 gm.

This was a medium which had been found well adapted to luxuriance of growth in agar and gelatin cultures. Third, 14 species of fungi were used for the inoculations, including all in the complete

list except *Mucor stolonifer*, *Penicillium glaucum*, *P. bicolor*, *Stysanus stemonites*, and *Trichoderma Koningi*. New cultures had been made of all of these and it was made certain that the spores were all viable and the cultures all pure. Inoculations were again in duplicate, giving four cultures of each fungus, two in N-free media and two in ammonium nitrate media. All the cultures were placed in the laboratory, protected from the air only by cotton stoppers. Observations were made once a week from January 3 to March 11, about 68 days.

Good growth took place in all the cultures and there were good indications that all were pure according to the inoculations. This was shown first by the fact that the duplicates in every case were very closely similar. Second, each culture showed the peculiar culture characters belonging to the species, such as coloring the medium pink in the case of *Fusarium*, or the black mycelium in the case of *Hormodendron*. Third, the four that were afterward analyzed were submitted to microscopic examination, which showed characteristic spores and fructifications, although spores were only meagerly produced in the N-free cultures. The amount of growth in the N-free cultures as compared with that in the ammonium nitrate cultures, as it appeared to the eye, is shown in fig. 17. The mycelium in the former was very loose and flocculent and almost wholly submerged. To the eye, it appeared to spread through two-thirds of the liquid in some cases, but as will be seen in the analytical results, when dried its weight was extremely small. It is worthy of note also that this growth all took place within the first 2 or 3 weeks, after which it ceased entirely. The growth in the ammonium nitrate medium was very abundant. The mycelium soon reached the surface, where spores began to form abundantly. The growth continued for many weeks and finally resulted in a thick felt over the surface and a mass of mycelium filling the liquid.

Five of these cultures which gave signs of the largest growth in the N-free solutions were now taken for analytical examinations. The mycelium of each was collected on a dried filter and dried to a constant weight. The filtrate was evaporated as previously described and analyses were made of both mycelium and filtrate.

A control was also analyzed to show the amount of nitrogen present. The control was treated exactly like the others except that it was sterilized immediately after inoculation.

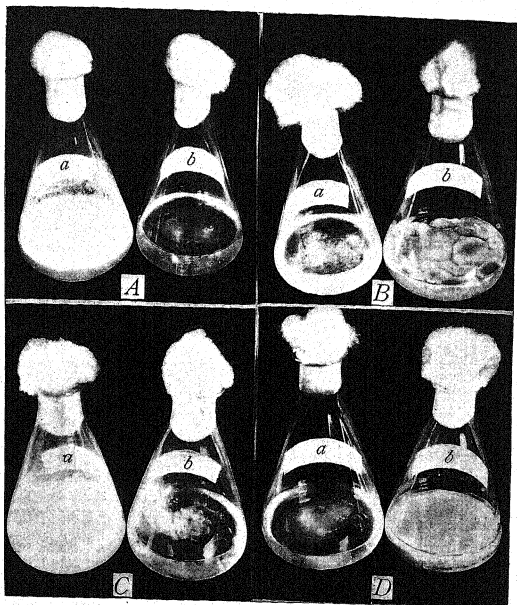


FIG. 17.—A, *Fusarium* (sp. ?): a, on ammonium nitrate medium; b, on nitrogen-free medium.

B, *Acrostalagmus cinnabarinus*: a, on nitrogen-free medium; b, on ammonium nitrate medium.

C, *Verticillium chlamydosporium*: a, on ammonium nitrate medium; b, on nitrogen-free medium.

D, *Pachybasium hamatum*: a, on nitrogen-free medium; b, on ammonium nitrate medium.

Table VI shows the results of this set of analyses. The nitrogen of a blank determination has been subtracted in each case from the total nitrogen obtained.

TABLE VI  
DEVELOPMENT PERIOD 68 DAYS

No.	Fungus	Dry wt. of mycelium mg. <i>a</i>	N of mycelium mg. <i>b</i>	N of filtrate mg. <i>c</i>	Total N of culture mg. <i>d</i>	N fixed (total control) mg. <i>e</i>
1	Control inoculated with Myceliophthora .....	.....	.....	0.49	0.49	.....
2	Control inoculated with Hormodendron .....	.....	.....	0.42	0.42	.....
	Av. for control .....	.....	.....	0.45	0.45	.....
3	Myceliophthora .....	6.0	0.49	0.42	0.91	0.46
4	Hormodendron .....	7.1	0.42	0.28	0.70	0.25
5	Hormodendron (dup.) .....	9.4	0.07	0.21	0.28	-0.17
6	Pachybasium .....	5.0	0.14	0.42	0.56	0.11
7	Pachybasium (dup.) .....	5.6	0.07	0.42	0.49	0.04
8	Acrostalagmus .....	8.6	0.28	0.14	0.42	-0.03
9	Acrostalagmus (dup.) .....	9.0	0.21	0.28	0.49	0.04
10	Fusarium .....	6.0	0.07	0.35	0.42	-0.03
11	Fusarium (dup.) .....	4.2	0.07	0.07	0.14	-0.31

From later work, it was believed that the dry weights of mycelium, given in column *a* of the table above, were a little too large. The reason for this seemed to be that the mycelia were not washed quite long enough to remove every trace of dextrose. Again, the nitrogen fixed, as indicated in column *e*, was high enough for nos. 3 and 4 to raise the question whether these two fungi might possibly have a little nitrogen-fixing power. To test these points further, cultures of these two fungi were run at the same time with later investigations, extending from April 17 to June 26. These cultures were treated exactly as in the above series, except that the phosphate content was increased to 3.0 gm., and the dextrose content to 45.0 gm. in 1000 cc. of the solution. This gave a higher nitrogen content for the controls, as well as for the filtrates of the cultures, since the glucose was found to contain some nitrogen. In washing the mycelia, 200 cc. of water was used instead of 100 cc. as in the earlier work. Table VII gives the results of these tests.

It is seen from this table that the amount of nitrogen gain in this case is well within the limit of error. The slightly higher value in the earlier work is believed to be due to less skilful use of the analytical methods. In harmony with this idea is the fact that *Myceliophthora* was the first one of the set which was analyzed. The results on the duplicate were thrown out altogether as worth-

TABLE VII

No.	Fungus	Dry wt. of mycelium mg. <i>a</i>	N of mycelium mg. <i>b</i>	N of filtrate mg. <i>c</i>	Total N of culture mg. <i>d</i>	N fixed (total control) mg.
12	Control.....			0.63	0.63	.....
13	Control (dup.).....			0.70	0.70	.....
	Av. control.....			0.66	0.66	.....
14	<i>Myceliophthora</i> .....	2.8	0.07	0.70	0.77	0.11
15	<i>Hormodendron</i> .....	3.0	0.14	0.56	0.70	0.04

less, due to a bad frothing in the distillation which evidently carried over some of the alkali. This difficulty was relieved by exchanging the 500 cc. distillation flasks for the 1000 cc. size, and using only stick zinc instead of granulated, after which no further difficulty of this kind was experienced. These results therefore give no foundation for nitrogen-fixation in these fungi, under the conditions of the investigation.

#### INVESTIGATION III

This investigation proceeded to work further with these same fungi, omitting *Acrostalagmus*, in nitrogen-free media, according to the method employed by TERNETZ (19), PENNINGTON (25), and others, using an apparatus for aerating the cultures by air drawn through strong potassium hydroxide and concentrated sulphuric acid. The apparatus is shown just as it was used in fig. 18. It consisted of six 500 cc. Erlenmeyer flasks connected up in sets of three in such a way that the air to each set passed over the following series: a U-tube of pumice, saturated with strong NaOH; a U-tube containing strong H<sub>2</sub>SO<sub>4</sub>; a wash bottle  $\frac{1}{2}$  filled with sterilized, distilled water; a tube 2 cm. in diameter containing a

germ-proof cotton stopper. The tube leading from the last divided into three, one entering each culture flask and dipping below the liquid. Each tube before entering the culture flask contained a bulb 2 cm. in diameter, which was also filled with cotton loosely packed. An exit tube passed out of each flask. The three from

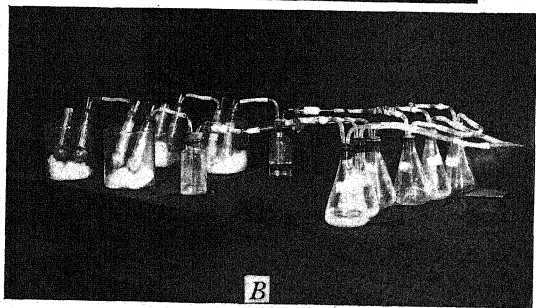
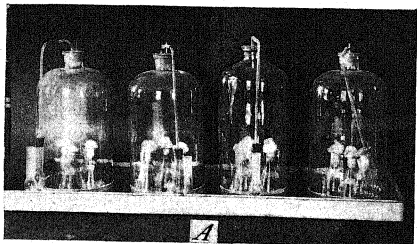


FIG. 18.—Apparatus for investigations IV (A) and III (B)

each set united into one, and these common tubes from each set united to form one tube which was connected to a filter pump. By the operation of this pump a slow stream of air was kept bubbling through all the cultures of both sets during the entire culture period. By means of clamps this stream was kept quite uniform

in all the flasks. Connections were made by means of new rubber stoppers and rubber tubing, which had been boiled in a 10 per cent solution of NaOH until perfectly clean. In addition, rubber stopper connections were sealed with paraffin. There was every indication that all the connections were air tight. The medium used for these cultures was made up as follows: \*

Conductivity water (ammonia-free).....	1000.00 cc.
Dextrose.....	45.00 gm.
Monopotassium phosphate.....	3.00 gm.
Magnesium sulphate.....	0.20 gm.
Calcium carbonate.....	0.10 gm.
Sodium chloride.....	0.02 gm.

Of this medium 100 cc. was supplied to each flask and then all apparatus except the U-tubes was sterilized in the autoclave at 115° C. Open ends were plugged with cotton during the sterilization and then connections were made as quickly as possible after removing these. Inoculations were made with the usual precautions, giving one culture for each of the four forms: *Hormodendron*, *Myceliophthora*, *Fusarium*, and *Pachybasium*. Two controls were prepared, one inoculated with *Myceliophthora* and the other with *Hormodendron*, and both sterilized immediately after inoculation. The latter of these controls became contaminated during the culture period and is therefore not considered in the analytical results. These cultures were allowed to develop from February 24 to May 4.

A perceptible growth occurred in all except the controls and examination, including microscopic, showed the cultures to be pure. The growth, while perceptible, was very scant. To the eye it appeared a little less than in those of investigation II, which stood in laboratory air. At the end of the development period previously mentioned, the cultures were disconnected and all sterilized in the autoclave. As soon as possible analyses were made as before, for mycelium and filtrate separately. The one uncontaminated control was also analyzed. The results are shown in table VIII.

From table VIII it is seen that no indication whatever is shown of nitrogen-fixation. The values for all the cultures are well within the limit of error of the analytical method.



In table VIII are also included some analyses of the medium to determine whether it took up an appreciable amount of fixed nitrogen by remaining exposed to the air of the laboratory. One culture was analyzed immediately after being made up, another after being kept during the last development period (70 days) under a bell jar, to which air could enter only over a U-tube containing concentrated  $H_2SO_4$ . A third was exposed for 70 days to laboratory air, which could enter freely through the cotton stopper. The analyses of these cultures show clearly that no fixed nitrogen, which was detectible by the method of analysis, was taken up from the air.

TABLE VIII  
DEVELOPMENT PERIOD 70 DAYS

No.	Fungus	Dry wt. of mycelium mg. <i>a</i>	N of mycelium mg. <i>b</i>	N of filtrate mg. <i>c</i>	Total N of culture mg. <i>d</i>	N fixation (total control) mg. <i>e</i>
1	Control. ....				1.18	
2	Myceliophthora. ....	3.0	0.07	1.26	1.33	0.15
3	Hormodendron. ....	1.3	0.07	1.19	1.26	0.08
4	Pachybasium. ....	3.1	0.00	1.12	1.12	—0.06
5	Fusarium. ....	1.5	0.07	1.26	1.33	0.15
6	Medium immediately after making up. ....				1.40	
7	Medium after standing exposed 70 days. ....				1.40	
8	Medium under bell jar 70 days. ....				1.50	

Table VIII shows a further point of some interest, viz., that the nitrogen which was shown to be present in the dextrose used is not available for the fungi, since approximately the same quantity is shown in the filtrates in which fungi have been cultivated as in the medium itself before inoculation. Furthermore, the spores introduced in inoculation do not add an appreciable quantity of nitrogen.

#### INVESTIGATION IV

This was carried out with cultures exactly similar to those in the previous investigation; but instead of being aerated, they were kept under bell jars from which all air was excluded, except that entering through a U-tube containing concentrated sulphuric

acid (fig. 18). The bell jars were sealed by means of vaseline to ground glass plates and water was kept in the pan enough to cover the lower edge of the bell jars at all times. These all developed some growth, not perceptibly different from the aerated cultures. Each fungus developed its specific characters. Analysis was made of only one of these cultures, but the dry weight of the mycelium was found for all. A control was also analyzed. The development period was the same as for those in investigation III (70 days). A comparison was also made in this investigation between the growth in ammonia-free water, prepared by another distillation of distilled water acidulated with sulphuric acid, and in the "conductivity water" obtained from the chemical laboratory. As seen from the dry weights in the table, no perceptible difference could be detected. Very little growth occurred in either. The data are given in table IX.

It is evident, by a glance at table IX, that these cultures showed no evidence of nitrogen-fixing power. The dry weights of the last four are too small to give any expectation of finding nitrogen by analysis. Repeated analyses of such amounts have shown no appreciable nitrogen present. Moreover, the one analyzed (no. 1) fully confirms this idea.

#### INVESTIGATION V

An attempt was made in this investigation to see whether fungi, which were supplied with sufficient combined nitrogen to start a good growth, would not under such conditions show a power to fix some free nitrogen. This was especially desirable, since the results of BERTHELOT (13), PURIEWITSCH (16), and LATHAM (20) have shown such decided nitrogen-fixation in such solutions.

The medium used was exactly the same as that employed in the last two investigations, except that varying amounts of ammonium nitrate were added. These solutions were made as follows: First, Erlenmeyer flasks of 150 cc. capacity were selected and cleaned ready for the solutions. Then a solution exactly like that used in investigation III was made up, using only half as much water, thus giving a solution of double the strength of all the constituents. This was called solution A. Then a solution of ammonium nitrate

was made in which exactly 16 gm. of the C.P. salt were dissolved in ammonium-free water. The salt for this was of course weighed out with the greatest accuracy on the analytical balance. The solution was then made up to exactly 1000 cc. in a volumetric flask, and was called solution *B*. Cultures were all made in duplicate. First, 25 cc. from solution *A* and 25 cc. from solution *B* were drawn off, by the use of an accurate burette, into duplicate flasks. This gave 50 cc. cultures, each of which contained exactly 0.40 gm. of pure ammonium nitrate, or approximately an *M*/10 solution. Then 50 cc. of solution *A* were accurately drawn off into a volumetric flask of 250 cc. capacity. This flask was then filled to the mark with ammonia-free water, thus giving a solution

TABLE IX

CONTROL, 100 CC., STERILIZED AFTER INOCULATION, 1.32 MG. N

No.	Name of fungus	Dry wt. of mycelium mg. <i>a</i>	N of mycelium mg. <i>b</i>	N of filtrate less control mg. <i>c</i>	Total N ( <i>b-c</i> ) mg. <i>d</i>	Gain mg. <i>e</i>
1	Myceliophthora.....	3.0	0.07	0.08	0.15	0.15
2	Hormodendron (conductivity water).....	1.6	Not analyzed			
3	Fusarium (conductivity water).....	1.2				
4	Fusarium (non-conductivity water).....	1.0				
5	Pachybasium.....	1.6				

of  $\frac{1}{5}$  the strength of solution *B*. Two culture solutions were now made by accurately drawing off 25 cc. of this diluted solution of ammonium nitrate and 25 cc. of solution *A* into culture flasks. This gave an *M*/50 solution of ammonium nitrate. By the continuation of this dilution process, further culture solutions were made containing *M*/250, *M*/1250, and *M*/6250 respectively of ammonium nitrate, all solutions having the same quantities of the other constituents as in investigation III. This may all be seen in brief in table X.

These cultures were sterilized and inoculated in the usual way and then allowed to stand in the laboratory without special protection from the air. During the final sterilization, a slight change

took place in the M/10 and in the M/50 media, a change which was more marked in the former. The cloudiness due to the undissolved calcium carbonate had entirely disappeared and the medium had become slightly brownish. This seemed to indicate some chemical change by which acid had been formed which dissolved the  $\text{CaCO}_3$ , and also a slight decomposition of the dextrose. Later analyses indicated that a loss of nitrogen in some form may have accompanied this change. In the other solutions no such change took place, indeed the cloudiness increased rather than decreased, due no doubt to loss of carbon dioxide during the boiling. This showed that the change above mentioned was due to the high concentration of the ammonium nitrate.

TABLE X  
CULTURES IN VARYING QUANTITIES OF AMMONIUM NITRATE AS INDICATED

No.	Mol. wt. concentration	Amount in 1000 cc. gm.	Amount in each culture (50 cc.) mg.	Calculated nitrogen mg.
1	M/10.....	8.0	400.0	140.19
2	M/50.....	1.6	80.0	28.04
3	M/250.....	0.32	16.0	5.61
4	M/1250.....	0.064	3.2	1.12
5	M/6250.....	0.0128	0.64	0.22

Two fungi were used for these cultures: *Fusarium* and *Myceliophthora*. Analyses were made of the latter only. The cultures were allowed to develop from April 17 to June 5, or 48 days. Vigorous and characteristic growth took place in all the cultures of both fungi.

The *Fusarium* produced the characteristic pink coloration of the medium, the degree of color depending on the amount of growth. At first the M/250 showed the best growth, but later this was surpassed by the M/50, and finally the best growth was in the M/10, although M/10 and M/50 showed but little difference. As previously stated, no analyses were made of this form.

The growth of *Myceliophthora* differed in the fact that the best growth both at the first and at the end was in the M/250 solution. Also the mycelium, at least in the higher concentrations, developed a small amount of purple color on the surface of the mat, while the

liquid itself was made yellowish brown. In the three higher concentrations a thick botryoidal mat was developed.

At the end of the growth period, all of the cultures of *Myceliophthora* were submitted to analysis to determine the amount of nitrogen in the mycelium and in the filtrate. The M/50 culture solution was analyzed for nitrogen content as a check on the purity of the ammonium nitrate. The medium without ammonium nitrate was also analyzed. The mycelium in each case was analyzed by the nitrate-free method, while the nitrate method was used for all the filtrates. The results are all incorporated in table XI.

TABLE XI  
DEVELOPMENT PERIOD 48 DAYS

No.	Concentration	Dry wt. of mycelium mg. <i>a</i>	N in the mycelium mg. <i>b</i>	N in the filtrate less medium (12) mg. <i>c</i>	Total N ( <i>b</i> + <i>c</i> ) mg. <i>d</i>	N supplied as $\text{HN}_3\text{NO}_3$ mg. <i>e</i>	N gain mg. <i>f</i>
First series							
1	M/10.....	98.0	5.41	130.85	136.26	140.19	-3.94
2	M/50.....	148.1	7.51	20.08	27.59	28.04	-0.45
3	M/250.....	242.6	4.91	0.70	5.62	5.61	0.01
4	M/1250.....	76.5	1.40	-0.14	1.26	1.12	0.14
5	M/6250.....	26.6	0.70	-0.21	0.49	0.22	0.27
Duplicate series							
6	M/10.....	98.6	5.19	130.71	135.90	140.19	-4.29
7	M/50.....	166.6	7.58	19.66	27.24	28.04	-0.80
8	M/250.....	257.2	5.19	0.42	5.62	5.61	0.01
9	M/1250.....	76.4	1.26	0.00	1.26	1.12	0.14
10	M/6250.....	25.4	0.42	0.00	0.42	0.22	0.20

11 M/50 control, not inoculated, 27.80 mg. N.

12 Medium without  $\text{NH}_4\text{NO}_3$  (50 cc.), 0.70 mg. N.

It is difficult to find any evidence whatever of nitrogen-fixation in these results, even though the combined nitrogen enabled the fungus to begin a vigorous growth. The nearest suggestion of evidence appears in analyses 4 and 5, where the mycelium seems to have gained a slight amount of nitrogen. In the same analyses the filtrates show a slight loss. Whether there is significance in this or whether it is just a coincidence in the errors of two analyses seems hard to say, since the amounts are so small as to give one little confidence in their importance. Furthermore, the duplicate

analyses show still smaller gains. In any case, these results could only show that the fungi under these conditions had been able to use a very small amount of the combined nitrogen, which the analyses of controls has shown to be present in the dextrose. All preceding evidence of this investigation has been against this idea, and we are strongly inclined to hold to the idea that these results are due to slight variations in the accuracy of the method of analysis.

Another interesting and perhaps important fact may be seen from the last table. The amount of nitrogen present in the mycelium is not proportional to the dry weight. This point was suggested earlier in the examination of mycelia grown in nitrogen-free media, where the hyphae looked somewhat shriveled and starved, as if the protoplasm lacked some necessary constituent. It appears here that the mycelium has the power of taking up a higher amount of nitrogen than it really needs for the best growth, judging best growth by the amount of dry weight, for in the two higher concentrations a larger percentage of nitrogen is shown, even though the dry weight is much less than in the M/250 concentration. The amount of nitrogen is also proportionally higher for the M/10 than for M/50. This holds for both series of cultures. If we take the averages of the two series, the per cents of nitrogen in dry weight of mycelium are approximately as follows: M/10, 5.5 per cent; M/50, 5 per cent; M/250, 2 per cent; M/1250, 1.8 per cent; M/6250, 2 per cent. Or, stated more generally, the fungus will assimilate about 2 per cent of its dry weight of nitrogen when this is supplied in such quantities that the fungus can use all that is present, but when the nitrogen is in excess of what the fungus can use, then a larger percentage is assimilated, running as high as 5.5 per cent. The evidence seems too scanty to make certain whether such a generalization would hold in all cases. Why this larger percentage of nitrogen is present in the solutions of higher concentration does not seem apparent. It may be that the presence of nitrogen compounds in excess causes the fungus to use these instead of carbon compounds, which are used in greater quantity when the nitrogen supply is limited. This investigation can only raise this question, and suggest its value as a subject worthy of further physiological investigation.

The loss of total nitrogen in the M/10 and M/50 cultures has been referred to. This may have been due, as already suggested, to some chemical action which gave off some compound of nitrogen during the sterilization. The dissolving of the calcium carbonate and the slight change in the color of the culture solutions suggested this, but no positive evidence of such nitrogen loss was obtained. Another possible explanation might be that nitrogen in some form was given off as a waste product during the metabolism of the growing fungus. However, no evidence of such a hypothesis was observed. This might raise another interesting question for further investigation.

#### DISCUSSION OF RESULTS IN RELATION TO NITROGEN-FIXATION

As a result of this series of investigations, it may be said that we find no evidence whatever which seems to justify a belief in the power of any of the forms investigated to fix free nitrogen under the conditions used.

The question of why such conflicting results regarding nitrogen-fixation by fungi have been reported by different investigators still remains. This investigation was not carried on to settle this question, but rather because it was hoped that by investigating a large number of forms, and especially those in the soil, some fungi might be found which certainly had the nitrogen-fixing power. There seems no reason a priori why fungi, as well as bacteria, should not have such power, and certainly, reasoning from analogy, there seems much reason to expect it. However, we have been wholly disappointed in this expectation as a result of this series of investigations. Furthermore, while it was not designed to work over forms previously studied, nevertheless, negative results are given here for one form, *Hormodendron cladosporioides*, for which positive results have been reported by others. Both FROELICH (11) and PURIEWITSCH (16) found appreciable nitrogen-fixation for this fungus on both nitrogen-free and nitrogen-poor media. We have been entirely unable to confirm these results.

It seems difficult to explain the widely conflicting results. It may be easy, perhaps, to discard entirely such results as those of LATHAM (20), in which, as pointed out by PENNINGTON (25),

6 similar 50 cc. cultures, having about the same quantities of combined nitrogen supplied to them, are reported to fix quantities of nitrogen all the way from 44.9 to 193.6 mg. This discrepancy is even more striking if we use the figures in column 10 of her "table I," which represent total amounts of nitrogen fixed. Here the results for the same 6 cultures as those above referred to vary between 33.3 and 205.1 mg. LATHAM's explanation that this is due to passing beyond the optimum or critical point in the supply of combined nitrogen in the culture hardly seems satisfactory, especially with the scant evidence at hand on this point. Furthermore, the great excess in the nitrogen gain over that reported for fungi by any other investigator, great even when compared with bacteria, would seem to require creditable confirmation before being finally accepted.

It might also be possible to reject some of the earlier work when methods of sterilization, inoculation, and pure cultures were so poorly perfected. However, the same cannot be said of the painstaking work of FROELICH (11), TERNETZ (19), and others, where accurate methods are reported and analytical data are fully given. It must be admitted, at the same time, that the weight of negative evidence is increasing, and it seems likely that few if any fungi will be found to have nitrogen-fixing power, unless, perhaps, it may be in the case of the mycorrhiza forms, regarding which more evidence is much needed.

This investigation does not seem to add weight to the explanations suggested by PENNINGTON of the conflicting results on this question, namely, that difficulties with the Kjeldahl method of analysis have led to variations in reports, and that differences in the strains of fungi used, suggested by THOM's (33) work, might have led to different results. As seen from previous data of this paper, the method of analysis seems capable of giving a limit of error which is far less than the differences in the results of different investigators. Such accuracy, however, could not be depended upon except in the hands of a skilled chemist, or at least one who had acquired considerable practice with the method. As to the second explanation, a good deal more work needs to be added to that of THOM along the line of modification through different culture



conditions. While this question was only incidental in this investigation, the experience with these fungi under quite a variety of culture conditions makes it hard to think that this can be a satisfactory explanation. Variations did, indeed, occur to some extent, but in all cases each form retained its distinct species characteristics, and showed no signs of nitrogen-fixation under any of the conditions.

If nitrogen-fixation is finally established for any fungous forms, it seems probable that the character of the nutrient material supplied may be found to be an important factor. It is not felt that this investigation supplies sufficient data along this line to permit final conclusions. There is also the possibility, as already suggested, that the nitrogen-fixing power may be found to be confined wholly to the mycorrhiza forms.

#### IV. Summary of results

This series of investigations would seem to justify the following generalization of results:

1. Many species of fungi live habitually in the soil, carrying out their life history there, either in whole or in part. A considerable number of these have been found, so far, only in the soil.
2. These fungi are, at least to quite an extent, uniform in different soils, and, unlike the bacteria, appear to be rather uniformly distributed at different depths, at least as low as 14 cm.
3. Tillage and manuring seem to produce little change in the number or kind of these fungi. This conclusion is not regarded as final.
4. These fungi may be cultivated and isolated as pure cultures, without interference from bacterial growth, by the use of 20-30 per cent of gelatin in the culture medium.
5. None of the forms studied, including at least 14 species, shows any power of assimilating free nitrogen when grown in nitrogen-free media under the conditions of these investigations. *Myceliophthora* and probably *Fusarium* show no such power even in nitrogen-containing media.
6. *Myceliophthora* when growing in nitrogen-containing solutions assimilates different proportions of nitrogen in different con-

centrations of the nitrogen compound. The nitrogen assimilated is approximately 2 per cent of the dry weight of the mycelium in all concentrations where the fungus is able to use all the nitrogen, in this case up to and including  $M/250$ . In higher concentration, where the nitrogen is in excess of what the fungus can use, the amount of nitrogen assimilated increases up to 5.5 per cent in the case of the  $M/10$  concentration. Also, the optimum growth as indicated by dry weight occurred where the fungus could use all the nitrogen, in which case the amount of nitrogen assimilated was 2 per cent of the dry weight.

7. The amount of combined nitrogen taken up from the air, by cultures standing exposed, does not seem to be sufficient to make appreciable difference in their nitrogen content, either in nitrogen-free or in nitrogen-containing media.

8. These fungi do not seem to be able to use nitrogen in all its forms, since analysis failed to show that they could use that present in the dextrose of the culture medium.

9. The Kjeldahl method of analysis is capable of a degree of accuracy which will reduce the limit of error very near to 0.1 mg. for each determination in analyses involving very small quantities of nitrogen. In analyses involving larger quantities of nitrogen, the error may be reduced to 0.3 of 1 per cent.

10. A very perceptible growth of mycelium is possible in practically nitrogen-free media, but in such cases the nitrogen content is found by analysis to fall within the limit of error of the method. Furthermore, the mycelium shows a starved, shriveled condition, as if deficient in some necessary element. In these cases, mycelia having a dry weight of 3-6 mg. gave amounts of nitrogen within the limit of error. Conversely, this may be something of a qualitative index of nitrogen-fixation, for, when the dry weight of mycelium is not more than 6-8 mg., there is little or no probability of nitrogen-fixation.

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## PHRYMA LEPTOSTACHYA L., A MORPHOLOGICAL STUDY

THEO. HOLM

(WITH PLATES VIII-X)

At an early date placed as the sole member of "Ordo CXLVI: Phrymaceae," the monotypic genus *Phryma* has been regarded, nevertheless, as representing a mere tribe of the Verbenaceae by BENTHAM and HOOKER, and until very recently by all American authors. As may be seen from SCHAUER's excellent description, the structure of the fruit ("caryopsideus monospermus") and of the seed ("exalbuminosum, embryon rectum, radicula supera cet."), besides the peculiar calyx ("superiore [labio] tripartito laciniis subulatis apice reduncis, inferiore [labio] brevissimo bifido"), these characters are much too distinct for admitting the genus among Verbenaceae, even as an anomalous genus. For many years the family Phrymaceae was thus ignored, until it became reestablished by BRIQUET<sup>2</sup> in a paper dealing with the anatomy of Phrymaceae, Stilboideae, Chloanthoideae, etc.; and further, in the treatment by this same author in ENGLER and PRANTL'S *Natürliche Pflanzenfamilien*.

Only one species is known, *P. leptostachya* L., but a few varieties have been described by BRIQUET. These are *parvifolia* (Rafin.) Briq., from the Allegheny Mountains, and preserved in the herbarium of DELESSERT; *inciso-crenata* Briq., cultivated in the garden of Ventenat; and *lanceolata* Briq., from Arkansas and Pennsylvania. The geographical distribution is quite remarkable, the species occurring in Canada (New Brunswick, Ontario), in the Atlantic States southward to Florida, and also in the Himalayas and in Japan; the variety *lanceolata* occurs with the type in Japan.

Notwithstanding the fact that the internal structure, of the mature plant only, has been described by BRIQUET, there are still

<sup>1</sup> SCHAUER, J. C., in DE CANDOLLE'S *Prodromus* 11:520. 1847.

<sup>2</sup> BRIQUET, J. I., *Mém. Soc. Phys. et Hist. Nat. Genève* 32:1894-97.

some points worthy of consideration drawn from the seedling and compared with the mature stage. Moreover, the origin of the secondary tissues in the stem deserve attention, besides the general structure of the root system.

### The seedling

In its native haunts seedlings of *Phryma* are difficult to be found. Being a truly sciaphilous plant and developing only a small number of seeds in proportion to the size of the plant, *Phryma* depends on dispersing its seeds, or better the fruits, by animals. The long, slender teeth of the persistent calyx are hooked at the tip, and are likely to adhere to fleece and clothing like a bur. Thus the fruits become scattered, and the seedlings are so very much like those of other sylvan types with opposite leaves that they may be mistaken for entirely different genera, of Labiatae and Compositae, for instance, when their cotyledons have dropped.

Fig. 1 represents the seedling (natural size) of *P. leptostachya*, and it is noticed at once that the cotyledons are hypogeic, and that they remain inclosed within the seed, surrounded by the thin pericarp. There is only a very short hypocotyl, and the primary root (*R*) is relatively short; a much longer secondary root (*r*) soon develops from beneath the insertion of the cotyledons and between them, as may be seen in fig. 2 (*r*). In the axils of the cotyledons buds are already visible at this very young stage (*B* in fig. 2), but they remain dormant during the first season. The aerial shoot of the seedling consists of a long, cylindric, glabrous, and erect epicotyl (*Ep.* in fig. 1), and a few short pubescent internodes with leaves of approximately the same outline as those of the mature plant. Characteristic of the seedling of *Phryma*, therefore, are the hypogeic cotyledons, the long epicotyl, and the early development of a secondary root, exceeding the primary in length and thickness. By this structure the seedling is easily distinguished from the corresponding stage of most of the other sylvan types with opposite leaves; for in the Compositae, for instance, the cotyledons are epigeic; and among the Labiatae, *Collinsonia*<sup>3</sup> is the only North American

<sup>3</sup> Compare Medicinal plants of North America. Merck's Report. New York. April 1909.

genus known to germinate with hypogeic cotyledons. As for the seedlings of the Verbenaceae, the cotyledons are epigeic in the genera examined by LUBBOCK<sup>4</sup> (*Verbena*, *Lippia*, *Clerodendron*, *Callicarpa*, *Stachytarpheta*, and *Raphitamnus*).

### The subterranean organs of the mature plant

An examination of the subterranean portion of the mature plant (fig. 3) shows that the primary root has faded away completely, but is replaced by several strong secondary roots, which are brownish, somewhat fleshy in texture, and which traverse the ground horizontally, all developed from the lowermost, very short internode of the floral shoot. Furthermore, the presence of several axillary buds (*B*) is observed, covered with opposite, scalelike leaves; one of these buds is much larger than the others, and from this a new floral stem will be produced during the following season. The subterranean stem portion of *Phryma* thus merely represents a pseudo-rhizome with a few short, persistent internodes, a few over-wintering buds, and a secondary system of roots. Similar to the seedling, the aerial shoot of the mature plant commences with a long internode preceding a few shorter ones, terminated by the long, very slender, loosely flowered spike. There is, therefore, a great resemblance between the habit of the seedling and that of the mature plant, with the exception of the early disappearance of the primary root in the latter.

### The root

None of the roots, neither the primary root nor any of the secondary roots of the seedling or of the mature plant, contain fungal hyphae, otherwise not infrequent in plants growing in shady localities; and in none of the roots was secondary increase to be traced beyond the stele. Root hairs abound, and a distinct exodermis with the cell walls suberized, and with foldings on the radial walls, was observed in the secondary roots, but not in the primary. The cortical parenchyma is homogeneous, compact, filled with starch in the secondary roots only. The endodermis is thin-walled,

<sup>4</sup> Contribution to our knowledge of seedlings. London. 1892 (p. 367).



and the pericambium consists of a single stratum with no indication of developing cork or secondary cortex. But inside the pericambium the stele soon increases in thickness in the usual way, and already at the seedling stage. While no pith was observed in the primary root, this tissue is well represented in all the secondary roots, attains quite a considerable width in mature specimens, and contains deposits of starch.

We have thus in *Phryma* two very distinct types of roots: nutritive roots, represented by the primary root; and a combination of contractile and storage roots, as shown by the secondary roots with contractile exodermis and starch deposits in cortex and pith.

#### The aerial stem

In seedlings and in young, purely vegetative shoots the internodes are cylindric, while in mature plants the stem becomes obtusely quadrangular and bisulcate to sharply 4-winged between the flowers. Furthermore, the internodes, at least in mature specimens, are more or less nodose, the node appearing some distance from the insertion of the leaves; these nodes are generally purplish, while the other parts of the stem are green. Similar nodes occur in various other plants, as among Labiatae, Scrophulariaceae, Polygonaceae, Acanthaceae, Caryophyllaceae, etc., and they may be located either directly at the insertion of the leaves or some distance therefrom. According to RÜTZOU,<sup>5</sup> the structure of the nodes in these families differs from that of the slender portion by the presence of more collenchyma, and of a less developed stereome; this author explains the function of these nodes to consist in facilitating the bending of the stem, when such is necessary. In *Phryma*, however, the internal structure is almost identical throughout the internode, including the swelling, but the function seems to be the same as observed by RÜTZOU.

The stem structure of *Phryma* in general is very uniform in young as well as in mature specimens, in the basal as well as in the apical internodes. The cuticle is thin and smooth in the subterranean stem portions, but becomes gradually thicker and longi-

<sup>5</sup> Bot. Tidsskr. Kjöbenhavn 12:248. 1880-1881.

tudinally wrinkled in the internodes above. The epidermis is thin-walled, and shows two types of hairs, pointed and glandular. The pointed hairs consist of a few cells in one row, covered with a granular cuticle; while the glandular hairs have a two-celled head borne on a single foot-cell, or on a long stalk of several cells in a single row; the sessile glandular hairs are especially frequent on the basal internodes, while the long-stalked hairs abound in the inflorescence. The pointed hairs are frequently curved to almost hooked, and occur especially on the upper internodes. Within the epidermis are a few (one or two) continuous strata of collenchyma, but only in the aerial internodes, not in the subterranean. The cortical parenchyma attains its greatest width in the short internodes of the pseudo-rhizome, where it consists of about 20 thin-walled compact layers; no crystals and no deposits of starch were observed in any part of the cortex.

The endodermis is present throughout the stem, but it is not always distinct, since the Casparyan spots are sometimes difficult to detect; besides, the individual cells are uniformly thin-walled, and of the same shape and lumen as the adjoining cortical parenchyma; moreover, the endodermis does not contain starch.

Bordering on the inner face of the endodermis is the so-called pericycle, relatively poorly developed in *Phryma*; as a closed sheath of stereids this tissue occurs only in the inflorescence, when the fruits have matured; in the pseudo-rhizome it is represented merely by parenchyma with isolated strands of stereome; while in the other internodes it is either uniformly thin-walled or interspersed with a few stereids, but with no regularity.

The herbaceous stem of *Phryma* does not increase much in thickness, and beyond the formation of secondary mestome strands no other secondary tissues were observed. The primary mestome strands are thus readily visible in all the internodes, and they are strictly collateral; there are 6 in the cylindric epicotyl of the seedling, and they are arranged in two broad groups; in the mature plant the mestome strands constitute 4 broad strands, one in each angle of the quadrangular stele. These primary strands contain leptome, cambium, and short rays of hadrome, in which the young vessels, reticulated and scalariform, are much wider than the

primordial, spiral and annular. An endoxyle (fig. 7) is quite well represented, especially in the subterranean internodes as well as in the swollen portion of the upper internodes. Between these primary mestome strands in the various stem portions (of seedlings and mature individuals) there are several strata of secondary mestome, that is, leptome and thick-walled libriform, but with no vessels.<sup>6</sup>

It is interesting to trace the origin of these tissues, the leptome and the libriform, and it might be stated at once that only the leptome develops from the pericycle, and only sometimes. What seems to be more important for the development of these secondary tissues in *Phryma* is undoubtedly the peripheral portion of the primary parenchymatic or medullary rays, which is still meristematic and capable of producing new tissues. Fig. 8 shows a small part of the interfascicular tissue, taken from the base of a very young lateral inflorescence, and here may be seen the thin-walled, large-celled endodermis (*End*), inside of which are small strands of young leptome, some perhaps developed from the pericycle itself, while others have developed in the tissue inside, the primary parenchymatic ray. As may be readily seen from this drawing, there is no real indication of any definite sheath such as a pericycle of one or of several strata. There is, on the other hand, a very distinct meristematic tissue in the periphery of the primary parenchymatic (medullary) ray giving rise to leptome, sometimes bordering on the endodermis, or some distance from it. In other words, this particular section (fig. 8) does not show the derivation of the secondary leptome from the pericycle alone, but from a meristematic tissue within the endodermis, a tissue of several homogeneous strata.

<sup>6</sup> SANIO (Bot. Zeit. 1863:101) proposed the term "libriform": "einfache (d. h. ungetheilte) bastartige Holzfasern oder Holzzellen, fibrae sive cellulae libriformes simplices. Um einen kurzen Ausdruck zu gewinnen werde ich dieselben Libriformfasern, und das daraus bestehende Gewebe Libriform nennen." HABERLANDT (Physiol. Pflanzenanat. 1896:138), however, calls attention to the fact that the distinction between libriform and stereome depends upon the different location of these tissues, and more so than in respect to their structure. SANIO's "libriform" applies to the stereids in the hadrome, and it is customary, therefore, to use this term for mechanical cells (stereids) that are developed inside the cambium, and stereome for those outside. However, from a physiological point of view, it seems unimportant whether such mechanical cells are located inside or outside the cambial zone, hence the distinction between libriform and stereome is not well founded except topographically.

In the internodes of the seedling, this structure, as described above, occurs in the apical internode, and with no indication, so far, of any development of secondary hadrome inside the leptome. To trace this, the origin of the secondary hadrome, we must examine the next internode, the one below the apical, shown in fig. 9. Here are seen the innermost strata of the cortex (*C*) and the endodermis (*End*); also, on the left side of the section, a part of one of the primary mestome strands (*M*) has been drawn, consisting of leptome (*L*) and a short ray of vessels. The broad interfascicular tissue consists, on the other hand, only of several well defined leptomatic strands, and of a tangential cell-division within the large, thin-walled parenchyma inside the leptome. There is actually no indication of any distinct pericycle in this section either, but there is certainly a very pronounced indication of the presence of a meristem in the periphery of the primary parenchymatic (medullary) ray. The result of these tangential cell-divisions within the leptome may be seen in fig. 10, which has been drawn from the epicotyl of the seedling. It consists of the development of thick-walled libriform in such a way that the innermost tangential cell wall becomes lignified, and very rapidly so, while the outermost is still meristematic, so as to give rise to another libriform cell in the same radius as the first developed. There is no distinct ring of cambium, therefore, since the innermost cell wall of each cell, which divides tangentially, becomes lignified almost at once, while the outermost remains active so as to yield another cell to the secondary hadrome, and always in the same radius.

In comparing the structure of these internodes of the seedling with that of an old internode of a mature specimen, which is drawn in fig. 15, we notice the continued growth of the interfascicular hadrome (*H*) as many radial layers of thick-walled libriform, while the leptomatic strands are about the same, and located in thin-walled parenchyma; no pericyclic sheath seems to be differentiated in this section either. This internode (fig. 15) was from the aerial portion of the stem, and examining the structure of the pseudo-rhizome, we notice a marked difference (fig. 16) consisting in the development of a partly stereomatic pericycle outside the primary mestome strands (*s* in fig. 16). As shown in this figure,

there are two thin-walled, parenchymatic strata between the endodermis (*End*) and the leptome (*L*), interspersed with stereids, and these two strata evidently represent what various authors have interpreted as a heterogeneous pericycle. Still another structure of this same pericycle may be observed in the oldest internodes of the inflorescence, that is to say, when the fruits have matured; in these there is a very distinct sheath of two layers of stereids surrounding the leptome, hence representing a homogeneous, in this case a purely stereomatic, pericycle.

The stereids inside the endodermis are thus only visible in the old, subterranean internodes of the main stem, and in the oldest internodes of the inflorescence, when the fruits have matured. But in all the other internodes, whether of seedlings or of mature plants, there is no pericyclic stereome, and barely any indication of a parenchymatic pericycle either. Moreover, the origin of the secondary mestome strands in *Phryma* cannot be attributed to the activity of a pericycle, but, as has been demonstrated, these secondary strands of leptome and thick-walled libriform arise from the meristematic, peripheral strata of the primary parenchymatic (medullary) rays.

Inasmuch as the origin of the secondary mestome strands, or, to be more exact, the interfascicular strands, seems to be different when we compare a number of dicotyledonous, especially herbaceous, stems, it might be appropriate to present a brief abstract of the history of the pericycle. The term "pericycle" was proposed by VAN TIEGHEM<sup>7</sup> and applied to the parenchyma, of one or several layers, which he found in the stem between the endodermis and the mestome strands, as well as in the roots between the endodermis and the outer face of the leptome, and between the endodermis and the protohadrome vessels. To the activity of the pericycle VAN TIEGHEM ascribed the origin of the interfascicular arches of cambium with the subsequent development of secondary mestome strands. MOROT,<sup>8</sup> in describing the pericycle in general,

<sup>7</sup> Sur quelques points de l'anatomie des Cucurbitacées. Bull. Soc. Bot. France 29:280. 1882.

<sup>8</sup> Recherches sur le péricycle ou couche périphérique du cylindre central chez les Phanérogames. Ann. Sci. Nat. Bot. VI. 20:249. 1885.

calls attention to the fact that while the pericycle is always present in roots (frequently called pericambium), it is not always to be found in stems; on the other hand, he agrees with VAN TIEGHEM in respect to the function of the pericycle. GÉRARD<sup>9</sup> and HÉRAIL<sup>10</sup> question the assertion of VAN TIEGHEM in respect to the function of the pericycle in stems, and describe a number of cases where the interfascicular tissues develop independently of the pericycle. Moreover, HÉRAIL describes some cases of secondary mestome developing in the pith (Melastomaceae and Campanulaceae). Finally, HABERLANDT<sup>11</sup> makes no use of the term pericycle in stems, but only in roots; and in reference to the secondary increase in stems, he speaks of a "cambium ring" being the direct product of a "procambium," and of an interfascicular cambium owing its existence to a meristem in the primary parenchymatic (medullary) rays.

In *Phryma* the presence of stereids is actually the only distinct indication of a pericycle, as scattered strands in the pseudorhizome, or as a closed sheath in parts of the inflorescence. The origin of these stereids I have been unable to detect, but it may be sought in the outermost layers of the leptome, and rather so than in any particular tissue, such as a pericycle for instance, inside the endodermis. Therefore, I am most inclined to believe that *Phryma* lacks a pericycle; in any case, the interfascicular tissues appear to develop independently of it, especially the libriform. However, the term "pericyclic" is quite convenient to use when describing the stereome strands between the endodermis and the leptome, because they are "peri" "cyclic," even if we do not always regard them as representing a pericycle as defined by VAN TIEGHEM, that is, a constant sheath of parenchyma from which the secondary tissues arise. It seems also worth while to compare this pericycle of stems with the well known pericambium of roots, where it represents the peripheral tissue of the stele, and typically so.

Returning to the stem of *Phryma*, the central part of the stele

<sup>9</sup> Passage de la racine à la tige. Ann. Sci. Nat. Bot. VI. 15:1881.

<sup>10</sup> Recherches sur l'anatomie comparée de la tige des Dicotylédones. Ann. Sci. Nat. Bot. VII. 2:1885.

<sup>11</sup> Physiologische Pflanzenanatomie. Leipzig. 1896.

is occupied by a broad, thin-walled pith, which is hollow in aerial internodes, but solid in the subterranean internodes, where it contains deposits of starch, but no crystals. As mentioned above, the nodes show no other modification of structure than an enlargement of the parenchymatic tissues, cortex and pith; partly also of the hypodermal collenchyma; furthermore, in these nodes the pericycle is very incomplete and without stereids; also the development of libriform goes on more slowly, so that several strata of cambium may be observed between the primary mestome strands.

### The leaf

As indicated by the varieties described by BRIQUET, the foliage of *Phryma* exhibits several quite distinct forms, some of which may be found on the same plant, if we examine the leaf-pairs from the basal to the apical internodes of seedlings as well as of adult specimens. The leaf is described by GRAY<sup>12</sup> as "ovate, acuminate, coarsely serrate." A little more is given by TORREY,<sup>13</sup> who calls them ovate, but distinguishes between the lower ones ("abruptly narrowed at the base, and furnished with long petioles") and the upper ones ("nearly or quite sessile"). SCHAUER describes the leaves as "ovato-oblonga, in petiolum longum attenuata, acuminata, grosse crenato-serrata"; and finally, the Himalayan plant is said by HOOKER<sup>14</sup> to have "ovate or ovate-lanceolate" leaves. As may be seen from the drawing of the seedling (fig. 1), the shape of the lowermost leaf-pair is broadly ovate and abruptly narrowed at the base, while the leaves above are elliptic. In fig. 4 I have drawn a leaf of a specimen from the mountains of Virginia, and the entire foliage of this specimen showed this same outline. Fig. 5 shows a leaf of a specimen from Ohio, in which the uppermost three pairs showed this outline, while the basal pairs were much broader. Finally, fig. 6 shows a leaf of a Japanese specimen, in which all the leaves were ovate and with the margin crenulate. Among some other specimens examined, I found the upper leaves of a specimen

<sup>12</sup> Synoptical flora of North America. Second Edition. 21:334. 1886.

<sup>13</sup> Natural history of New York. 1843 (p. 53).

<sup>14</sup> Flora of British India 4:561. 1885.

from Nippon elliptic, while the lower ones were ovate-oblong; all the leaves of a specimen from Kansas were lanceolate; while all the leaves of a plant from Georgia were ovate. The leaf margin was observed to vary to the same extent from crenate to serrate, even on the same individual. It is thus somewhat difficult to define the typical leaf-form in *Phryma*; it varies much, but the ovate outline may be the fundamental one.

The internal structure is bifacial, the stomata being confined to the dorsal face, and the chlorenchyma being differentiated into a ventral palisade tissue and a dorsal pneumatic tissue. On both faces of the blade the cuticle is thin and smooth except above and below the veins, where it is wrinkled. The epidermis is a little thick-walled where it covers the veins, and the cell-lumen is somewhat wider on the ventral face than on the dorsal (fig. 17). Viewed in superficial sections, the lateral cell walls of the epidermis are undulate on both faces of the blade, and the stomata lack subsidiary cells. Short, almost sessile, glandular hairs (figs. 12, 13) abound on both faces of the leaf, and, as may be seen from the drawings, the head is two-celled. Besides these glandular hairs there are also, and especially on the ventral face, some long, pointed, multicellular hairs (in one row) with cuticular pearls. The chlorenchyma consists of a single layer of very short and plump palisade cells (*P* in fig. 17) covering three strata of an open pneumatic tissue (*P*<sup>+</sup> in fig. 17): in superficial sections this pneumatic tissue shows intercellular spaces of considerable width (fig. 18).

The mechanical tissues are poorly represented, there being only a few hypodermal layers of collenchyma above and below the midrib and the secondaries, and also the leptome of the midrib is supported by an arch of thin-walled stereome (*S* in fig. 19). A broad, thin-walled water-storage tissue surrounds the midrib, but there is no endodermis; the lateral veins, on the other hand, are surrounded by green parenchyma sheaths (fig. 17). All the mestome strands are collateral, and the median is the broadest. The petiole, when examined just beneath the blade, shows exactly the same structure as the midrib, except that it contains two very thin mestome strands, one on each side of the median. Finally, it may be mentioned that the throat of the corolla of the flower is not



naked as stated by SCHAUER, but covered with numerous hairs, glandular (fig. 11) as well as pointed (fig. 14).

### Summary

*Phryma* represents a sciaphilous type, well marked by the structure of the ample leaf blade, with low palisade cells and very open pneumatic tissue; also by the poor development of the mechanical tissues in the stem as well as in the leaf. The vegetative reproduction is confined to the few buds on the short pseudo-rhizome, and the roots produce no shoots. For dispersing the seeds the plant is well equipped by the hooked teeth of the persistent, reflexed calyx.

BROOKLAND, DISTRICT OF COLUMBIA

### EXPLANATION OF PLATES VIII-X

FIG. 1.—Seedling of *Phryma leptostachya*: the dotted line indicates the surface of the soil; *Ep*, epicotyl; *Cot*, the achene with inclosed cotyledons; *H*, hypocotyl; *R*, primary root; *r*, secondary root; natural size.

FIG. 2.—Basal portion of same seedling: *B*, buds in the axils of cotyledons; other letters as above; magnified.

FIG. 3.—The pseudo-rhizome of a mature specimen: *S*, base of stem; *B*, bud which will produce a floral shoot in the following year;  $\times 2$ .

FIG. 4.—Leaf of a specimen from Virginia: two-thirds natural size.

FIG. 5.—Leaf of a specimen from Ohio: two-thirds natural size.

FIG. 6.—Leaf of a specimen from Japan: two-thirds natural size.

FIG. 7.—Cross-section of a subterranean internode showing the endoxyle;  $\times 480$ .

FIG. 8.—Cross-section of the basal internode of a lateral inflorescence: *End*, endodermis; *L*, secondary leptome;  $\times 744$ .

FIG. 9.—Cross-section of one of the apical internodes of the seedling: *C*, cortex; *M*, hadrome of a primary mestome strand; *P*, pith; other letters as above;  $\times 744$ .

FIG. 10.—Cross-section of the epicotyl of a seedling: *L* and *H*, interfascicular leptome and libriform; other letters as above;  $\times 744$ .

FIG. 11.—Glandular hair from the throat of the corolla;  $\times 600$ .

FIG. 12.—Glandular hairs from the leaf;  $\times 600$ .

FIG. 13.—Same;  $\times 600$ .

FIG. 14.—Pointed hair from the throat of the corolla;  $\times 600$ .

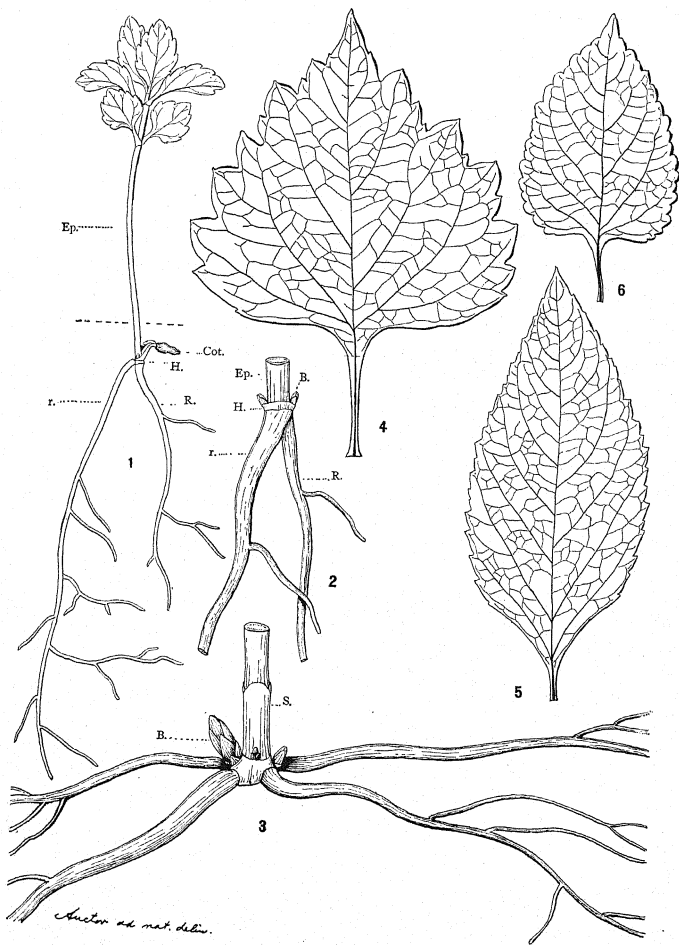
FIG. 15.—Cross-section of an old internode of a mature specimen: *L* and *H*, interfascicular leptome and libriform; the other letters as above;  $\times 600$ .

FIG. 16.—Cross-section of a subterranean internode of a mature specimen: *S*, stereomatic pericycle; *Camb*, cambium; other letters as above;  $\times 744$ .

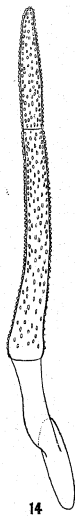
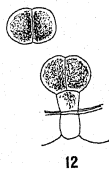
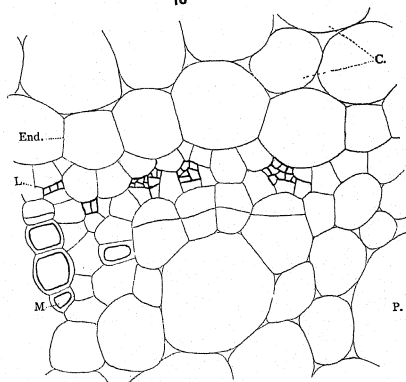
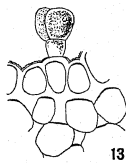
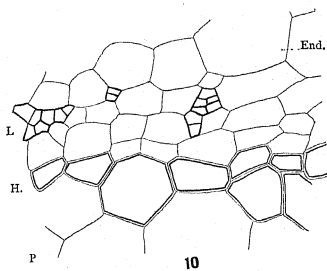
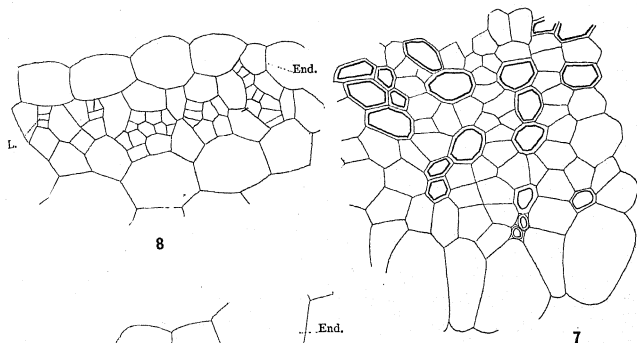
FIG. 17.—Cross-section of lateral part of leaf blade: *Ep*, ventral epidermis; *Ep*+, dorsal epidermis; *P*, palisade tissue; *P*+, pneumatic tissue;  $\times 600$ .

FIG. 18.—Pneumatic tissue of leaf, superficial section;  $\times 360$ .

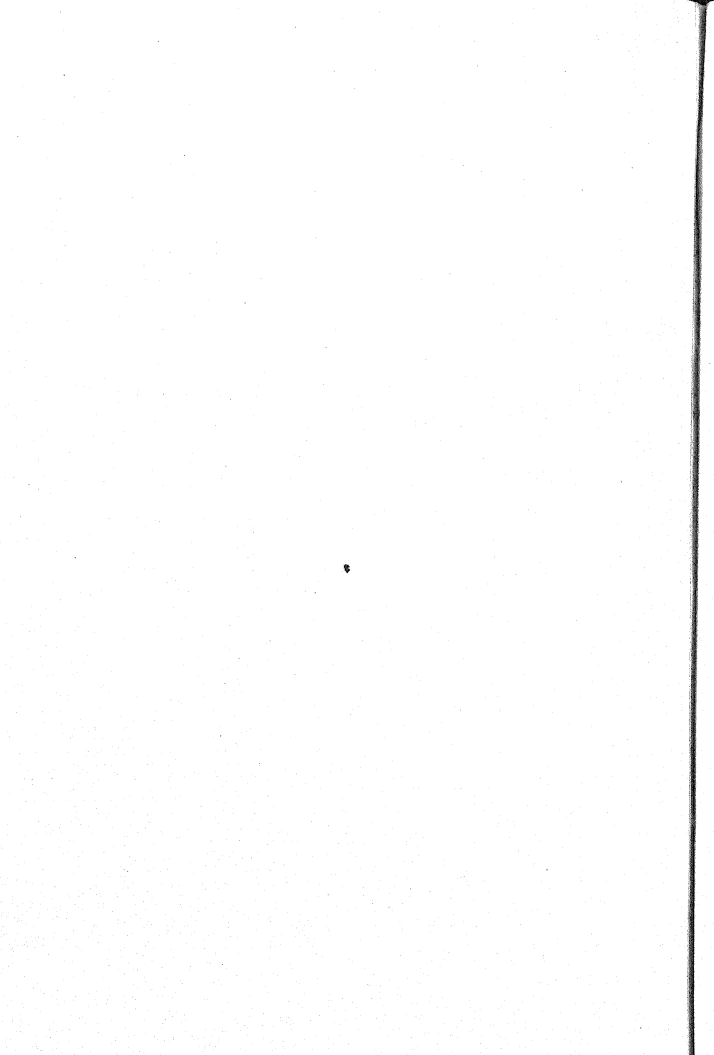
FIG. 19.—Cross-section of part of midrib of leaf: *S*, stereome; *L*, leptome; *H*, hadrome;  $\times 600$ .

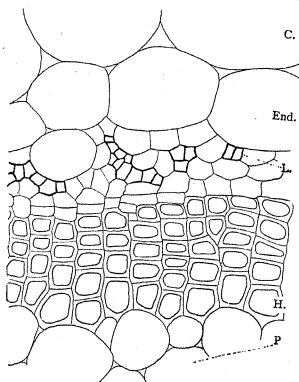




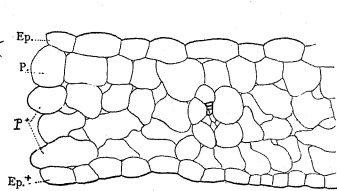


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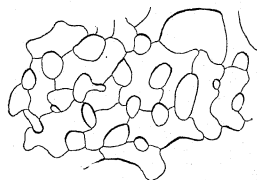




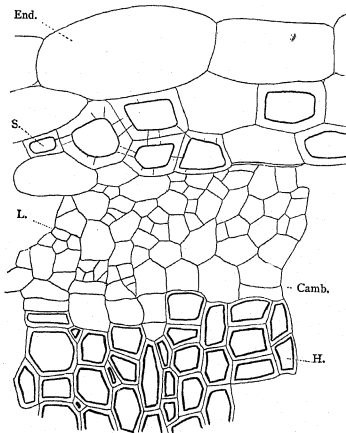
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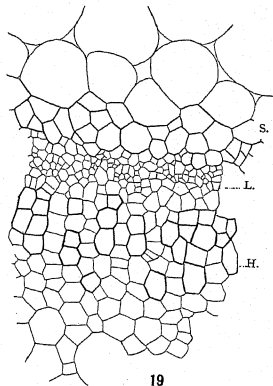
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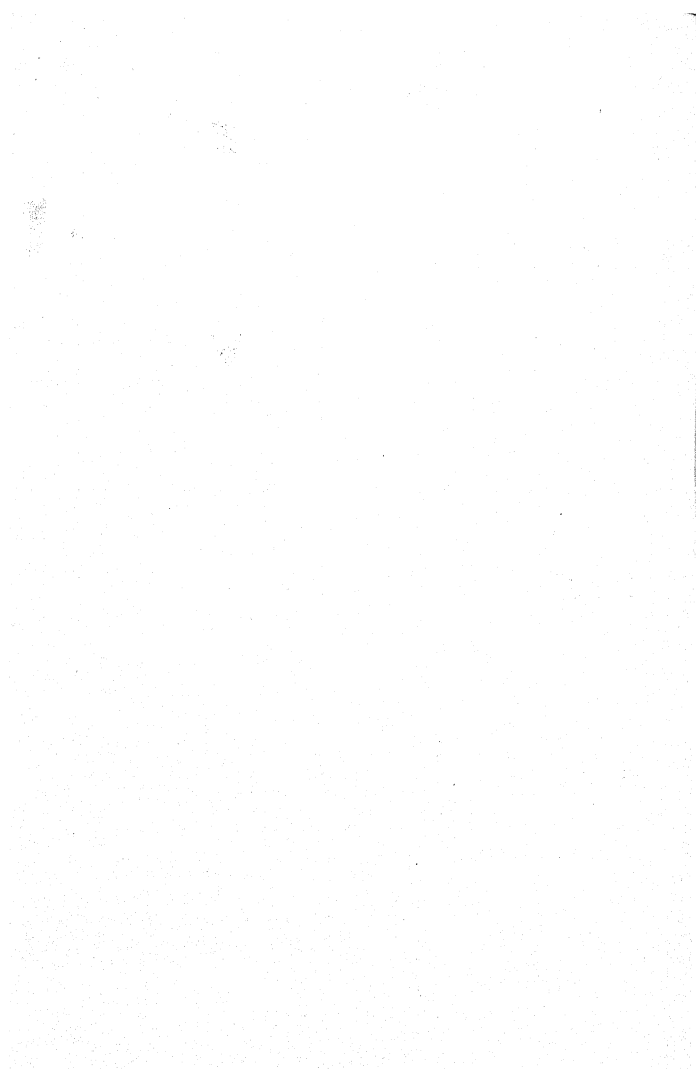
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## NUCLEAR DIVISION IN SPIROGYRA CRASSA

M. L. MERRIMAN

(WITH PLATES XI AND XII)

Although the cytology of *Spirogyra* has been investigated probably more than that of any other alga, it still offers a fruitful field of inquiry. The varying accounts, beginning with STRASBURGER'S paper in 1882 as to the chromatic or non-chromatic nature of the parts of the nucleus and the rôle they play in karyokinesis, have left many questions open.

*S. crassa* was chosen for this investigation on account of the large size of the nuclei, spindles of which can be detected with a magnifying glass. Specimens were fixed in weaker chromacetic, Flemming, and Bouin mixtures. Sections were cut 3-5  $\mu$  in thickness. When spindles of the metaphase were sectioned, 3-5 sections of one spindle were obtained. The stains used were safranin and gentian violet, Heidenhains hematoxylin with iron alum. Extended observations upon other species with smaller nuclei, but more easily studied in the living cells because of their greater transparency, have aided in making the interpretations given in this paper. In order to interpret sections of the large nuclei of *S. crassa* more satisfactorily, nuclei in different stages of division were also dissected from the filaments and from them whole mounts made. This dissection was necessary as nuclei in *S. crassa* are surrounded by a substance ramifying in the cell and so rendering them somewhat opaque. This condition, as well as that of their being obscured by the close winding of the chromatophores, prevented the adequate study of their division in the living cells.

In the living cells and in the mounted specimens three membranes can be distinguished clearly, one surrounding a central dense spherical body, a second surrounding a less dense larger body containing the first, and lastly a third which is continuous with the suspensors which in turn are continuous with the cord on which Boubier (3) has demonstrated the pyrenoids are dependent. These

membranes when not easily seen in the living cells were readily demonstrated by the use of methylene blue.

The initiation of division is marked by the enlargement of the nucleus and the gathering of granules in a vertical plane about the nucleus in the short axis of the cell. A study of the nucleus at this stage reveals great variations in the appearance of the central body, the so-called nucleolus, and the surrounding part, nuclear-plasm. The periphery of the enveloping portion may consist of a fine network, the interstices of which are occupied by coarse or fine granules (fig. 5). Or in place of the granules a deeply stained but vacuolar material may form the junction of the threads (figs. 1, 2, 4). This vacuolar-like material seen at the junction of the threads is in appearance like that seen in the chromosomes in late metaphase and anaphase. It also resembles that seen in *Allium*, for here the material is likewise thickened at the corners, suggesting four granules inclosing a vacuole.

The central body may appear from the staining to be of uniform density (figs. 1, 2, 3), or it may be more or less vacuolar (fig. 4). In a later stage the central body may appear to disintegrate into deeply stained granules, while in the meantime the network becomes coarser and accumulations at the interstices of the deeply stained material more pronounced. This at times may consist of filamentous (fig. 3) instead of granular or vacuolar masses, hence tending to support GRÉGOIRE's view (8) that there is no strict morphological distinction between chromatic granules and an achromatic substratum, and that in nuclei both an alveolar and reticular structure may obtain. These variations are due doubtless, as DIGBY (5) suggests in *Gallonia*, to the colloidal nature of the chromatic substance.

As the central body disintegrates (fig. 6), the space about it becomes gradually clearer, coincident with the expansion of the central mass which now occupies several times the original space. The fact that the space previously occupied by the network becomes clear, leads to the conclusion that network and nucleolus have become centralized in the spherical mass (figs. 7 and 8) now consisting of granules and filaments of varying dimensions irregularly disposed. All observers of nuclear division in *Spirogyra* are

practically unanimous in the view that the nucleolus is partly or wholly transformed into the substance of the spindle; the disagreement is as to whether the network furnishes any of that substance. The chromatic nature of this network is shown in figs. 2 and 3.

The nuclear membrane still intact shows that as yet none of the bodies previously seen in the space has been cast into the cytoplasm, unless in solutes by diffusion. This was confirmed by the study of whole mounts (fig. 8). Hence we may conclude that the enlargement of the central mass is due to the inclusion of the substances of the network, all now showing an increased staining capacity. It is possible, as in fig. 6, where short filaments are seen distributed throughout the nucleus and superimposed on the central mass, that the latter as it disintegrates only contributes the granular material to the spindle.

With the enlargement of the central mass the evolution of the spindle proceeds. The suspensors increase in size, while streams of granules appear to penetrate the nuclear membrane and connect with the centrally lying mass (fig. 7). These later increase as the mass of chromatic material changes its shape from that of a sphere (figs. 6-8) to a cylinder (figs. 13-16). Now the streams of granules appear to cause the cylinder to swing so as to lie with its long axis parallel with the long axis of the cell. In the fully developed spindle nothing of a fibrous nature is to be perceived but lines of granules staining as the cytoplasm, and so always distinguished from the equatorial mass. These lines of granules terminate in granular masses not linearly arranged. The masses are later seen to envelop the daughter nuclei (figs. 34, 36).

In sections of late prophase two kinds of material, one staining more deeply than the other, can be seen occupying the nuclear space, the nuclear membrane still visible. The more deeply stained material is in the form of short filaments (fig. 12) or lumplike masses (figs. 13, 15). The chromatic material here with its dumbbell-shaped (figs. 13, 19, 20) or somewhat elongated granules often resembles that seen in precipitates secured by FISCHER when nucleic acid is precipitated with Flemming and chromic acid mixtures (7, p. 43). Also they may be reasonably compared with those in

fig. 2 (7, p. 34), secured when 20 per cent albumose is precipitated in a concentrated aqueous solution of sublimate. FISCHER has figured both of these bodies of unequal staining capacity, precipitated from the same substance and appearing as stages in division. The fact that they are secured by precipitation might indicate that in *Spirogyra* we have a similar case. The dumbbell-shaped bodies seen frequently in the centrally lying mass may be but stages in aggregation and not the beginning stages of division. They are probably the bodies described by BERGHS (1) and MITZGEWITCH (12) as the chromosomes, although in the species studied by these writers 12 chromosomes were found. As in *S. crassa* they are variable as to number and shape and in all cases far exceed the number of chromosomes heretofore ascribed to *Spirogyra*, there seems to be justification for considering them not chromosomes, but rather amorphous masses of chromatin material which will serve as centers for the absorption of the less dense surrounding material.

FISCHER (7) found that nuclein acid granules, as they did not become corroded by iron alum, could be stained only a light smoke gray with aqueous hematoxylin, but when inclosed in deuteroalbumose solution their aversion to corrosion by alum salt was overcome. This suggests that the clue to the difference in the staining capacity of the component parts of the central mass may be sought in analogous changes in the composition of the colloids.

Although both kinds of material, ordinarily in the form of a spherical mass, nearly fill the nuclear space, in nuclei fixed with Bouin's mixture this was not found as frequently as the condition where the nuclear space is filled with irregularly shaped bodies undifferentiated as to stain.

With the elongation of the nucleus, the nuclear membrane first dissolves at the longer axis where the protoplasm condenses. The mass of material now gradually condenses, changing from spherical to cylindrical, the deeply stained material accumulating more along the central axis. Here it may be seen to be composed of oblong bodies, when filaments are arranged so as to be seen endwise (fig. 17), or as filaments somewhat intertwined. In all cases an areolar space marks off the entire spherical mass from the spindle. The darker stained bodies are also distinguished from the others by

areolae. These bodies may be considered but as centers of condensation, where by absorption the finer granular matter is transformed to the denser state. The areola around the denser bodies, as well as that around the entire mass, may be of no morphological significance, as FISCHER (7) obtained, when staining precipitates of deutero-albumose, all stages between a deeply stained central point and an areola of perceptible diameter.

It was not until drawings had been made of the thin sections and until the second year of this investigation that it occurred to me to make whole mounts of the dissected nuclei. These presented so different an appearance from the thin sections as to warrant an entirely different interpretation of the stages which show striations of granules and longitudinal arrangement of the deeply stained bodies. When such nuclei in stages just before and up to the complete dissolution of the nuclear membrane are examined, a continuous spireme including both kinds of material can be seen distinctly (fig. 9). The coils of this spireme are connected by anastomosing bridges. The deeply stained bodies, which seemed irregularly disposed in the thin sections (figs. 10, 13), in the whole mounts appear to lie with the granules in a spireme; a spireme, hence, not homogeneous, but consisting of material longitudinally arranged and of varying density, representing the pachyneme stage described by investigators on other plants.

This spireme, however, appears to be not greatly dissimilar to that seen in *Allium* in the prophase. In *Allium* there was more regularity in the arrangement of the chromatin masses and linin forming the spireme. In *Spirogyra* (fig. 11) also an apparent longitudinal division of the spireme is present. This parallelism of the threads has been described by DIGBY (5) in *Galtonia*. In *Trillium*, GRÉGOIRE (8) has interpreted it as an apparent splitting caused by progressive alveolization of the chromosomes; in *Allium*, BONNEVIE (2) has attributed similar appearances as due to the chromatin gathering at the peripheral portion of the chromosome, forming a spiral coil within; while the writer (10) in *Allium* regarded the apparent split as the result of an aggregation of granules forming a quadripartite thread.

The great similarity of this spireme to that described by other

writers is further evidence that in *Spirogyra*, as in *Allium*, we have here but a stage preparatory to the organization of chromosomes, and all bodies seen in preceding stages are but amorphous precipitates, their great variation being due to the colloidal nature of the chromatic substance. But slight pressure on the cover glass of these whole mounts appears to destroy the spireme appearances; the material then appears, as in sections, to consist of dark bodies and fine granules. These dark bodies sometimes appear as oblong granules. Examination of many slides and of some where the centrally lying material has been slightly displaced by the knife (fig. 17) indicates that the bodies appear oblong only because the filaments are seen endwise. There is nothing to indicate a definite number of these darkly stained bodies which appear to be homologous with those termed chromosomes by MITZGEWITCH (12), WISSELINGH (13), BERGHS (1), and others. Their appearance as lying in a spireme in whole mounts and their subsequent behavior in relation to the finer granular material show them to be, with the other material, but parts of the as yet unorganized chromosomes.

Returning to the study of the sections, we find a tendency of the deeply stained bodies to gather at the equatorial part of the cylinder in longitudinal rows (fig. 16). An illustration of this may be seen in the first section of a spindle, where both kinds of material show this longitudinal arrangement. A second section, cut deeper in the same spindle and so taking in less of the chromatic material, does not show such a bewildering array of bodies. Here the spireme formation can be seen as distinctly as in the case of the whole mounts. It is also in this stage that there can be seen a beginning of a trend of both kinds of material to the poles. This orientation of the chromatic material to the respective poles of the spindle, without the intervention of centrospheres, suggests that at this period in karyokinesis the chromatin masses may have acquired electrical charges, thus bringing about a state of mutual repulsion. A sharp split or rift along the equatorial circumference of the spindle does not appear, but instead, the interior (figs. 20-22) and then the equator (fig. 23) becomes gradually cleared of material. As this clearing proceeds, it is coincident with an amalgamation (figs. 23, 24) of the two kinds of material. Amalgamation when observed in

living material appears to be due to the gradual loss of the more liquid portion streaming each side. This leaves the interior of the cylindrical-shaped spindle clear as amalgamation proceeds. When one looks down with low power upon a nucleus in this state, as in fig. 22, the mass appears to have separated, though no actual separation of the chromatic material has occurred; for use of higher power shows all the bodies still united in a spireme, although all the material has left the interior. Similar strepsinema stages in the whole mounts show that this amalgamated material is also in spireme formation (fig. 25), though no differentiation is to be seen but the line indicating its double nature. The general shape assumed by the spireme in its convolutions is flatter now than at the earlier stages. This flattening culminates in the elongation of its coils (fig. 26). As a result of these changes there is but one kind of material of intensive staining capacity, pointing to either pole. This amalgamation can be compared with that in *Allium* where the parallel threads made of granules fuse to form tubular chromosomes. In *Spirogyra*, as in *Allium*, the spireme evolves from substances of two staining qualities; in both, the amalgamated materials yield tubular chromosomes staining intensively with hematoxylin and anilin stains. Similar elongation of amalgamated filaments is shown by BERGHS (1). He did not observe a spireme, and as he terms the earlier indefinite deeply stained bodies the chromosomes, these amalgamated filaments he terms "pseudochromosomes." As their formation from irregular masses of chromatin by absorption and condensation proceeds to an organized spireme behaving in its entirety as the spiremes in *Allium* that segment into chromosomes, it is difficult to see why these amalgamated substances should be called "pseudochromosomes."

These coils of the spireme, now completely on the peripheral part of the cylinder, pull apart (figs. 26, 27). A definite transverse splitting does not appear, but instead there is a gradual elongation and constriction as of viscid masses. At last, attenuation brings about a separation of the chromatic strands, not at any definite dividing line, but at various points in the spireme. This was confirmed in the study of living, dividing nuclei of a more transparent species of *Spirogyra*. Further evidence that the spireme ruptures

at different points is to be found in the fact that chromosomes, as in fig. 26, are often stretched across, when most of the material is already near the poles. As they separate, the chromosomes are connected by an anastomosing network (fig. 30), a beginning of that seen in the developed daughter nuclei. Often, if not always, strands are left after the assembling of the chromosomes at the poles. These chromatic masses persist in the cell after the membranes of the daughter nuclei are formed. As they can be traced step by step to the chromatic material of the metaphase, they may rightfully be termed chromidia and not considered metabolic products deposited in the cytoplasm. Their frequent occurrence at this stage of division suggests that the casting out of such masses may be a normal phenomenon, and that these chromidia are active in the secretory or other functions of the cell.

DERSCHAU (4) observed in the leaf epidermis cells of *Berberis* chromatin protuberances from the nucleus beginning to turn green. He considers them chromidial substances laying the foundation of the chloroplasts. LEWITSKY also holds that the chromatophores develop from chondriosomes, although MEYER (11) considers his conclusions unsound, as his investigations led him to believe that chromatophores arise only through the division of other chromatophores. FARMER and DIGBY (6) describe in certain varietal and hybrid ferns the ejection from nucleus into cytoplasm of chromatic droplets during early stages of heterotypic mitosis and also during telophase. Future research may likewise connect in *Spirogyra* these chromidia with the origin of chromatophores and pyrenoids.

As the chromosomes elongate and assemble at the poles, a split lengthwise can be seen in each, in some more definitely than in others (figs. 28-30). Sections cut obliquely (fig. 27) show the tubular or hollow formation of the chromosomes; those cut transversely, the ringlike nature resolving later into the four thickened masses inclosing a vacuole. The number of these chromosomes could not be ascertained definitely, but it seemed to approximate 14, more rather than less. This would correspond with that found by KARSTEN (9) in *Spirogyra jugalis*.

As the chromosomes near the poles they become V-shaped (fig. 33). When condensation has sufficiently proceeded, the ma-



terial is seen to assemble more at the ends of the double V's, thus presenting the appearance of tetrads (fig. 28). The V-shaped chromosomes represent but the loops or portion of the loops of the original spireme now ruptured. Each V has the characteristic line as seen in the original spireme, showing it to consist of parallel threads of chromatin. The more deeply stained ends of the loop are homologous with the structures described by BERGHS (1) as the true chromosomes reappearing from the mass of "pseudo-chromosomes," an interpretation which seems unwarranted when their whole history is taken into consideration.

After rupture of the spireme, the chromosomes do not form daughter spiremes, but joined by anastomosing bridges of linin cohere loosely in a ring or disk (fig. 32, 33). These figures are not dissimilar to those of GRÉGOIRE (8) in *Trillium*, where each of anastomosed chromosomes after polar assemblage becomes by alveolization an elementary *reseau*, at once of an alveolar and reticular nature. The chromosomes in the ring or disk gradually shorten their loops, and approach each other more closely until a confluence of the chromatic material results in one to several vesicular masses (figs. 35, 36) lying within a granular material that formerly was at the ends of the spindle and into which the chromatin retreated. These masses are crossed by cavities, spherical or polyhedral. At this time the chromatic masses are surrounded by a clear space, around the margin of which the nuclear membrane begins to evolve (fig. 37).

### Summary

A summary of the results obtained that differ most from others published is as follows:

A. spireme originates from material derived from both nucleolus and nuclear network. The materials constituting this spireme are aggregations varying in appearance, in number, and in staining capacities.

These aggregations are not the chromosomes. They greatly exceed in number that published for chromosomes in any species of *Spirogyra*; although a comparative study of plates of other

investigators indicates that these are the bodies heretofore designated as chromosomes.

This spireme in the pachyneme stage is composed of deeply stained short filaments intermixed with material of a granular nature. There is evidence that this granular material was derived from the nucleolus, the filamentous from the nuclear network.

These two materials amalgamate to form one of intensive staining capacity. The amalgamated material retains the spireme form. This spireme as a whole is spherical, later elongates, becoming cylindrical. Cross-sections of the loops reveal their tubular structure.

This spireme does not appear to split either transversely or longitudinally, but separates at various points as would a viscid mass if pulled in opposite directions. Fourteen or more tubular chromosomes for each daughter nucleus result from the elongation of the coils of the spireme. These are not to be considered "pseudochromosomes."

At this stage and subsequently chromidia are discharged into the cytoplasm. It is probable that these chromidia are concerned in the development of pyrenoids.

There is no evidence throughout the karyokinesis of an equational division of autonomous bodies. The advantage of this form of division over direct divisions appears to lie in the opportunity for escape of the chromidia from the nucleus.

*Spirogyra crassa* does not in the behavior of its nucleus in karyokinesis present a unique case, for the stages can be homologized with similar stages in *Allium*, as typical of the higher plants.

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## EXPLANATION OF PLATES XI AND XII

All figures were sketched with an Abbé camera. The nuclei were stained with Heidenhain's hematoxylin and iron alum, or with safranin and gentian violet.

FIGS. 1-7.—Sections representing changes in nucleolus and nuclear network up to the invasion of the spindle fibers; nuclei fixed in Bouin fluids (figs. 1, 6), in chromacetic (figs. 2, 3, 5, 7), in Flemming (fig. 4); Leitz oc. 4 and 1/12 oil im.

FIGS. 8, 9.—Nuclei dissected from filaments and mounted without sectioning; fixed in chromacetic; Zeiss comp. oc. 12 and Leitz 1/12 oil im.

FIG. 10.—Section of stage similar to that in fig. 9; same fixing and magnification.

FIG. 11.—Whole mount of nucleus at later stage than in fig. 9; only central portion drawn to show change in shape of spireme; Leitz oc. 4 and 1/12 oil im.

FIG. 12.—Section of stage similar to that in fig. 11; fixed in Flemming; Zeiss comp. oc. 12 and Leitz obj. 6.

FIGS. 13-16.—Figs. 13 and 16 fixed in chromacetic, figs. 14 and 15 in Flemming; Leitz oc. 4 and 1/12 oil im.

FIG. 17.—Section fixed in chromacetic, in which some filaments are dislodged by knife; Zeiss comp. oc. 12 and Leitz obj. 6.

FIG. 18.—Section cut obliquely; fixed in Flemming; Leitz oc. 4 and obj. 6.

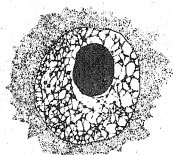
FIGS. 19-24.—Sections fixed in chromacetic (figs. 19, 20, 23, 24) and in Flemming (figs. 21, 22); Zeiss oc. 6 and Leitz 1/12 oil im.

FIG. 25.—Whole mount of stage similar to that in fig. 24; Zeiss oc. 12 and Leitz obj. 6.

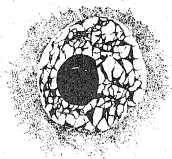
FIGS. 26-31.—Sections fixed in chromacetic (figs. 26, 27, 31) and in Flemming (figs. 28, 29, 30); Leitz oc. 4 and 1/12 oil im.

FIG. 32.—Whole mount, showing somewhat polar views of disks of chromosomes; Zeiss oc. 12 and Leitz obj. 6.

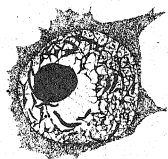
FIGS. 33-37.—Sections fixed in Bouin fluid (fig. 33), in Flemming (fig. 35), in chromacetic (figs. 36, 37); also a whole mount (fig. 34); Leitz oc. 4 and 1/12 oil im.



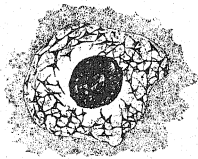
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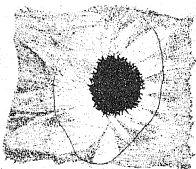
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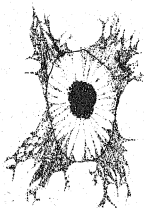
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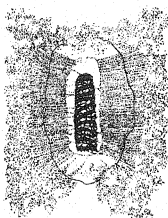
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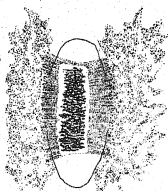
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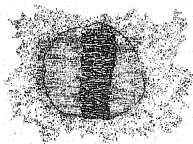
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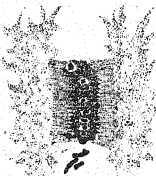
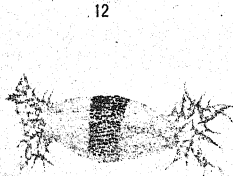
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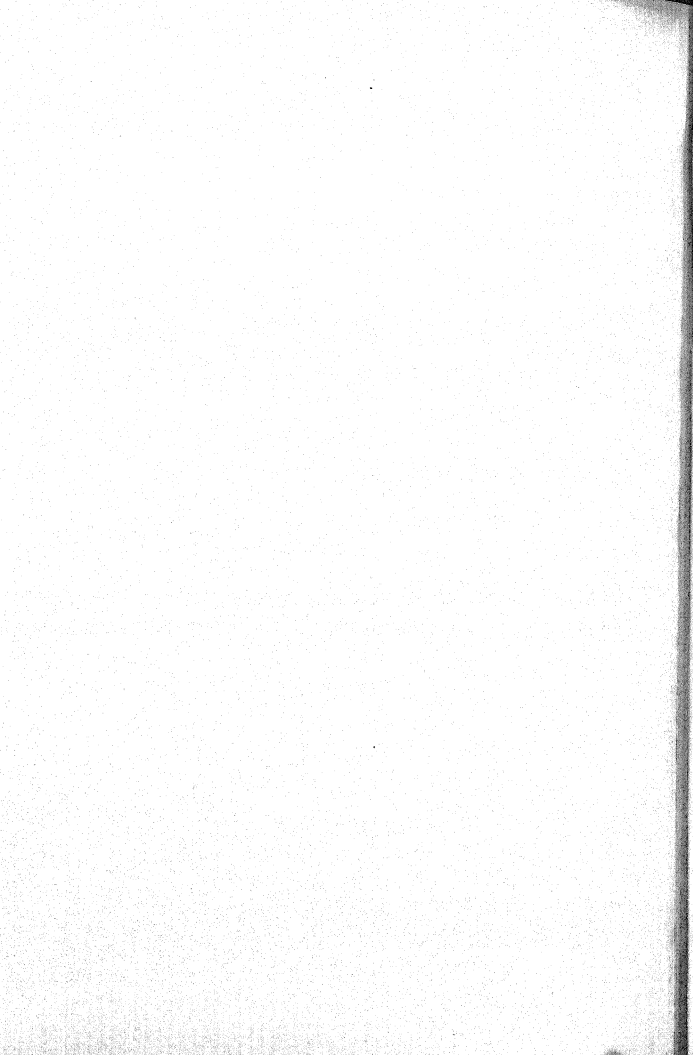


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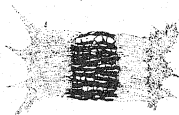
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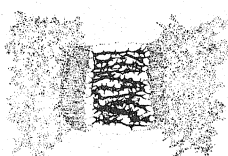
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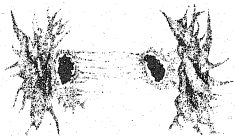
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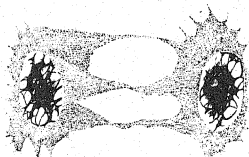
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## FILICES PURDOMIANAE

CARL CHRISTENSEN

(The ferns collected by Mr. WILLIAM PURDOM in 1910 in Shensi during the Arnold Arboretum expedition to northern China have been placed in the hands of Dr. CARL CHRISTENSEN of Copenhagen. His report upon them is found in the following paper.—C. S. SARGENT, *Arnold Arboretum*.)

### Alphabetical list of species

The species marked by an asterisk are probably new to the province of Shensi.

1. *Adiantum aristatum* Christ.—Tai-pei-shan, no. 79.
2. *A. erythroclamys* Diels.—Tai-pei-shan, no. 78.
3. *A. pedatum* L.—Tai-pei-shan, nos. 71, 80.
4. *Asplenium adiantum nigrum* L.—No. 74.
5. *A. Sarelü* Hook.—Nos. 73, 76.
6. *A. varians* Hook. et Grev.—No. 75.
7. *A. trichomanes* L.—Tai-pei-shan, no. 77.
8. \**Athyrium acrostichoides* (Sw.) Diels.—No. 49.
9. *A. Biondii* Christ.—Tai-pei-shan, no. 43.
10. *A. crenatum* (Sommerf.) Rupr.—Northern Chili, no. 64.
- 11a. *A. filix-femina* (L.) Roth.—No. 48.
- 11b. *A. filix-femina* (L.) Roth var. *multidentatum* (Döll).—No. 62.
12. \**A. Henryi* (Bak.) Diels.—Tai-pei-shan, no. 42.
13. \**A. mongolicum* (Franch.) Diels var. *Purdomii*, var. nov.—Nos. 13, 20.
14. \**A. Sargentii*, sp. nov.—Tai-pei-shan, no. 10.
15. *A. spinulosum* (Maxim.) Milde.—Tai-pei-shan, nos. 57, 58, 60.
16. (?) *A. subsimile* Christ.—No. 50.
17. *Blechnum eburneum* Christ.—WILSON no. 4723.
18. *Cheilanthes argentea* (Christ) Hook.—WILSON no. 4724.
19. *C. lanceolata*, sp. nov.—Tai-pei-shan, nos. 24, 25.
20. *Cheilanthes* sp.—No. 15.

21. *Coniogramme fraxinea* (Don.) Diels.—No. 82.
22. *Cryptogramma Stelleri* (Gmel.) Prantl.—Tai-pei-shan, no. 72.
23. *Cyclophorus pekinensis* C. Chr.—No. 91.
24. *C. Sheareri* (Bak.) C. Chr.—Tai-pei-shan, no. 97.
25. *C. taeniodes* C. Chr.—No. 83.
- *C. taeniodes* var. *furcata*.—No. 89.
26. *Cystopteris fragilis* (L.) Bernh.—No. 47.
27. \**C. moupinensis* Franch.—Tai-pei-shan, nos. 14, 63.
28. \**Doryopteris concolor* (Langsd. et Fisch.) Kuhn.—No. 16.
29. *Drynaria reducta* Christ.—No. 87.
30. *Dryopteris lacera* (Thunb.) C. Chr.—No. 68.
31. \**D. Linnaeana* C. Chr.—Nos. 35, 45.
32. \* (?) *D. marginata* (Wall.) Christ.—No. 67.
33. \**D. nipponica* (Franch. et Sav.) C. Chr.—Tai-pei-shan,  
no. 38.
34. \**D. oyamensis* (Bak.) C. Chr.—Tai-pei-shan, no. 88.
35. \**D. phegopteris* (L.) C. Chr.—No. 70; new to China proper.
36. *D. polylepis* (Franch. et Sav.) C. Chr.—Tai-pei-shan,  
nos. 37, 55.
37. \**D. Purdomii*, sp. nov.—Tai-pei-shan, no. 44.
38. \**D. sericea*, sp. nov.—Tsin-ling, no. 66.
39. *Gymnopteris bipinnata* Christ.—Tai-pei-shan, no. 28.
40. *Matteuccia intermedia*, sp. nov.—Tai-pei-shan, nos. 1, 8,  
9, 12.
41. *Microlepia Wilfordii* Moore.—No. 69.
42. \**Polypodium (Goniophl.) amoenum* Wall.—No. 85.
43. *P. (Gon.) subamoenum* Clarke var. *chinense* Christ.—  
Tai-pei-shan, no. 32.
44. \**P. (Pleopeltis) clathratum* Clarke.—Tai-pei-shan, no. 96.
45. *P. (Pl.) eilophyllum* Diels.—No. 90.
46. *P. (Pl.) lineare* Thunb.—Tai-pei-shan, no. 93.
- 46a. *P. (Pl.) lineare* var. *contortum* Christ.—Nos. 92, 94.
47. *P. (Pl.) oligolepidum* Bak.—No. 95.
48. *P. (Pl.) shensiense* Christ.—No. 84.
49. *Polystichum acanthophyllum* (Franch.) Christ.—Nos. 21,  
52 (in part), 54, 56, 34(?).
50. *P. aculeatum* (L.) Roth.—No. 61.

51. *P. Braunii* (Spenn.) Fée.—Tai-pei-shan, nos. 40, 51, 59.  
 52. *P. craspedosorum* (Maxim.) Diels var. *Giraldii* Christ (?).—  
 No. 52 in part.  
 53. \* *Polystichum gracilipes*, sp. nov.—Tai-pei-shan, nos. 17, 31.  
 54. *P. lachenense* (Hook.) Bedd.—Tai-pei-shan, no. 46.  
 55. *P. moupinense* (Franch.) Bedd.—Tai-pei-shan, nos. 36, 39,  
 53, 65(?).  
 56. \* *P. Thomsoni* (Hk.) Bedd.—Tai-pei-shan, nos. 22, 33(?).  
 57. *P. (Cyrtomium) falcatum* (L.) Diels var. *polypterum*  
 Diels (?).—Tai-pei-shan, nos. 81, 86.  
 58. *Pteris multifida* Lam.—Tai-pei-shan, no. 11.  
 59. \* *Woodsia lanosa* Hook. var. *attenuata*, var. nov.—No. 19.  
 60. *W. polystichoides* Eat.—Tai-pei-shan, nos. 23, 29, 41.  
 61. *Lycopodium annotinum* L.—No. 2.  
 62. *Selaginella sanguinolenta* (L.) Spr.—No. 27.  
 63. *S. Stauntoniana* Spring.—No. 26.

#### Remarks on some species with descriptions of the new forms

ADIANTUM ARISTATUM Christ, BOT. GAZ. 51:356. 1911.—  
*A. monochlamys* var. *latedeltoidea* Christ, Nuov. Giorn. Bot. Ital.  
 N.S. 4:88. 1897.—Tai-pei-shan, no. 79.

CHRIST has later identified his proposed variety with *A. Davidi* Franch.  
 (see Bull. Soc. Bot. France Mém. 1:62), but it differs from that species just  
 as the recently described *A. aristatum* does.

ADIANTUM ERYTHROCHLAMYS Diels.—Tai-pei-shan, no. 78.

Probably this is what CHRIST has named *A. monochlamys* typus *elongatus*  
 (Nuov. Giorn. Bot. Ital. N.S. 4:88. 1897). It differs from the typical *A.*  
*monochlamys* Eat. in the completely entire margins of its pinnae and in its  
 very large indusia.

ATHYRIUM MONGOLICUM (Franch.) Diels var. *Purdomii*, var.  
 nov.—Habit, magnitudine, rachi sursum complanata, alata  
 typo similis, differt: rhizomate vix repente, apice squamis atro-  
 brunneis nitidis linearibus longe acuminatis dense onusto; pinnis  
 basi aequalibus, i.e., lacinia anteriore vix aucta; laciniiis approxi-  
 matis fere quadratis apice truncatis leviter crenato vel obtuse

dentato; indusiis magnis persistentibus tenuibus, fere omnibus reniformibus, nonnullis breviter hippocrepiformibus, nullis rectis.

Shensi: Tai-pei-shan, PURDOM no. 13 (type); no. 20 is apparently the same.

**Athyrium Sargentii**, sp. nov.—Rhizomate erecto, squamis latis rufo-brunneis vestito. Stipitibus dense fasciculatis pallidis, basi nigrescentibus incrassatis, 2–3 cm. longis, nudis. Lamina lanceolata, 20 cm. circiter longa, medio 6–7 cm. lata, utrinque attenuata, tenuiter membranacea, laete viridi, bipinnatifida. Rachi gracili, supra sulcata et pilis pluricellularibus mollibus pubescente. Pinnis 15–20-jugis, sessilibus, recte patentibus, inferioribus reductis et reflexis, medialibus maximis, 3–3.5 cm. longis, vix 1 cm. latis, oblongis, basi vix auriculatis, ad costas costulasque utrinque sparse hirtis, profunde pinnatifidis. Lobis patentibus sinubus acutis separatis, integris vel leviter crenatis, apice obtusis vel truncatis. Venis 4-jugis, simplicibus, ascendentibus. Soris 3–6 in lobo, medialibus; indusiis brevibus (vix 2 mm. longis), lineari-ovatis vel ovatis, turgidis, pallidis, marginibus integris, persistentibus, nullis hippocrepiformibus, nullis reniformibus.

Shensi: Tai-pei-shan, PURDOM no. 10. This new species of the group of *A. acrostichoides* (Sw.) Diels stands between *A. Girdaldii* Christ and *A. mongolicum* (Franch.) Diels. It resembles the former, which I have not seen, in its basal scales, the inflated bases of the stipes, and in the shape of the indusia, but it is much smaller and its segments are not triangular-falcate. From *A. mongolicum*, which it resembles closely in size, cutting, and general habit, it differs in its basal scales, the rachis not being winged upward, and especially in the totally different indusia.

**Cheilanthes lanceolata**, sp. nov.—*Aleuritopteris* rhizomate breve crasso, apice squamis rigidis lanceolatis brunneis marginibus pallidis 3–4 mm. longis dense onusto. Stipitibus fasciculatis, ad 10 cm. longis, teretibus, strictis, fragillimis, atropurpureis, nitidis, squamis late-ovatis tenuissimis pallide brunneis deciduis instructis. Lamina lanceolata, ad 20 cm. longa, medio 5–6 cm. lata, versus basin paululum angustata, tripinnatisecta, pilis squamisque omnino destituta, pagina inferiore farina alba subdense obecta, superiore ut rachi glandulosa. Pinnis valde remotis, usque ad 10-jugis, infimis saepe abbreviatis, maximis 3–4 cm. longis, deltoideis vel deltoideo-

oblongis, sessilibus, obtusis vel acutis. Pinnulis 4-5-jugis, ovatis vel oblongis, obtusis, inferioribus late adnatis 1 cm. longis, 5-7 mm. latis, superioribus decurrentibus, versus apicem pinnarum confluentibus, profunde lobatis. Lobis ovatis, obtusis, maximis crenatis. Indusiis subcontinuis, leviter fimbriatis vel potius erosis, pallide-luteis.

Shensi: Tai-pei-shan, PURDOM nos. 24 and 25. A most distinct species of § ALEURITOPTERIS, characterized by the lanceolate shape of the lamina, which is entirely destitute of hairs and scales. In general habit it resembles not a little the Mexican *C. aurantiaca* (Cav.) Moore, although the pinnae are somewhat more divided. The stipe and rachis are very fragile.

CYSTOPTERIS MOUPINENSIS Franch.—Tai-pei-shan, nos. 14 and 63.

No doubt specifically different from *C. sudetica* A. Br.; the indusia are eglandulose.

DRYOPTERIS LACERA (Thunb.) O. Ktze.—No. 68.

Very typical. *A. filix-mas* var. *Giraldii* Christ, Nuov. Giorn. Bot. Ital. N.S. 4:94. 1897 is no doubt this species.

*Dryopteris Purdomii*, sp. nov.—*Lastrea* rhizomate (?), stipitibus ad 10 cm. longis stramineis gracilibus, supra bisulcatis et decidue hirtis. Lamina anguste lanceolata, 30-35 cm. longa, medio vix 5 cm. lata, utrinque attenuata membranacea, bipinnatifida. Rachi gracili straminea, pilis albis brevibus patentibus deciduis sparse pubescente. Pinnis inferioribus 2-3-jugis abbreviatis, 1-2 cm. longis, inter se 7 cm. remotis, medialibus maximis 3-4 cm. longis, basi 1.2-1.4 cm. latis, sessilibus, 3 cm. inter se remotis, e basi latiore versus apicem breviter acuminatum sensim attenuatis, plerumque curvatim erectis, supra pilis brevibus antrorsis praesertim ad costas costulasque setulosis, infra ad costas costulasque pilis patentibus albidis subdense hispidulis, marginibus ciliatis, ad alam 1 mm. latam pinnatifidis. Laciniis obliquis, triangulari-acutis, marginibus revolutis integris, basali superiore parum longiore. Venis indivisis, ca. 5-jugis, obliquis, basalibus marginem supra sinum acutum attingentibus. Soris medialibus, numerosis, exindusiatis, receptaculo piloso, sporangiis setosis.

Shensi: Tai-pei-shan, PURDOM no. 44. Most like a very narrow form of *D. brunneo-villosa* (Wall.) C. Chr., which it resembles in pubescence and in its triangular-oblong pinnae; but it is much smaller, and its setose receptacles and sporangia mark it clearly. *D. rufostraminea* (Christ) C. Chr. has similar pilose sori, but, according to the description, it is not very much like our plant.

**Dryopteris sericea**, sp. nov.—*Eudryopteris* rhizomate (?), stipitibus strictis, griseis, glabris, ad 10 cm. longis, basi squamis rufis tenuibus ovato-acuminatis integris dense onustis. Lamina ovato-oblonga, 25 cm. longa, 10–12 cm. lata, versus apicem pinnatifidum sensim attenuata, basi rotundata, textura crassa, opaca, in siccitate supra brunnea infra grisea, ubique pilis articulatis brevissimis in aetate omnibus deciduis dense griseo-tomentella, bipinnatifida vel potius subbipinnata. Rachi griseo-straminea, superne sulcata, in parte inferiore squamis brunneis minutis sparse vestita. Pinnis 7–10-jugis, adscendentibus, alternis, inferioribus 4–5-jugis breviter petiolatis, superioribus sessilibus, late-oblongis, maximis ad 6 cm. longis, 2–2.5 cm. latis, latere posteriore paullulum latiore saepe inaequilateralibus, ad apicem breviter acuminatum vel non raro obtusum serratum e medio sensim attenuatis. Segmentis secundi ordinis basalibus pinnarum inferiorum liberis, basi cordatis et utrinque auriculatis auriculis brevibus obtusis, sequentibus iis aequalibus, 1.5 cm. longis, 5 mm. latis, basi posteriore decurrentibus, anteriore auriculatis, marginibus crenatis vel interdum lobatis, superioribus ala 1 mm. lata connectis, omnibus obtusissimis. Venis furcatis vel bifurcatis, 6–8-jugis. Soris medialibus; indusiis reniformibus, rufis, subglabris, persistentibus. Sporangii glabris.

Shensi: North of Sian Fu, Tsin-ling Range, PURDOM no. 66. A most distinct fern, in its peculiar pubescence unlike all other species of *Eudryopteris*, to which subgenus it no doubt belongs. The whole frond is clothed with very short articulated hairs resembling those occurring in the subgenus *CTENITIS*; they are found equally throughout the surfaces and not mainly confined to the costae above as in *CTENITIS*; the costae are, like the veins, scarcely visible from the upper side. In venation, especially in the decurrent secondary veins, in color, indusium, and basal scales, our species agrees with most species allied to *D. filix-mas*; in general habit it resembles not a little *D. cristata*. The type specimen consists of three fronds, of which two are pubescent as described, while the third is absolutely glabrous, but otherwise perfectly similar to the two others; it is probably an older leaf with all the hairs fallen. As in several other species of the same relationship, the lower 2 or 3 pairs of pinnae are sterile.

GYMNOPTERIS BIPINNATA Christ in Lecomte, Not. Syst. 1:55. 1909.—*Gymnogramme Delavayi* Christ, Nuov. Giorn. Bot. Ital. N.S. 4:17. pl. 3. fig. 3 (non BAKER).—Tai-pei-shan, no. 28.

*Matteuccia intermedia*, sp. nov.—Species critica inter *M. struthiopteridem* et *M. orientalem* medium tenens, a priore quae habitu magnitudine similis, differt: paleis basalibus nigris, rachibus paleaceis, lamina longe stipitatis, versus basin breviter decrescente, pinnis lobatis nec profunde pinnatifidis, venis tertiariis circiter 5-jugis, pinnis fertilibus 6–7 cm. longis, 3–4 mm. latis recurvatis; a posteriore, quae venatione, colore, rachi paleacea similis, differt; pinnis angustioribus, inferioribus 2–3-jugis abbreviatis.

A most critical form, intermediate between *M. struthiopteris* (L.) Tod. and *M. orientalis* (Hook.) Trev. The sterile leaf resembles *M. struthiopteris* in size and in breadth of pinnae; it differs in (1) the long stipe, which is up to 20 cm. long, 0.5 cm. or more thick, broadly furrowed above, in the lower part and especially at base clothed with large (2 cm. long by 0.5 cm. wide), nearly black, glossy, and entire scales; (2) the lamina being very shortly attenuate downwards, the lower 2 pairs of pinnae only being somewhat abbreviated; (3) the rachis, like the costae of the pinnae beneath, clothed with several minute blackish scales; (4) the pinnae, which are not very close, linear, 10–12 cm. long by 1.5 cm. broad, incised not more than a third of the way down to the midrib into broad, obtuse lobes; basal lobes scarcely prolonged, but the lower one considerably broader and imbricating the upper side of the rachis, under surface pale, glandular with scattered yellowish glands; (5) the venation, the tertiary veins being very oblique, about 5 to a side, the lower ones curved upward and running to the sinus. *M. orientalis* has much broader and more deeply cut pinnae, the basal ones not at all abbreviated. Fertile leaf with stipe and rachis rather scaly beneath with light-brown, crisped scales; stipe 20–30 cm. long, lamina 40–50 cm. long, about 10 cm. broad; lower pinnae a little shortened, median ones 6–7 cm. long, 3–4 mm. broad, at first erect, later recurved from the erect base with hanging tips; costae scaly beneath, the veins distinctly seen on the upper side. Indusium apparently wanting.

As a whole, our new species is perhaps nearest to *M. orientalis*, resembling it in color, the scaly rachis, and especially in the large fertile leaves, but in general habit it is much more like *M. struthiopteris*. It is possible that *Struthiopteris orientalis* var. *brevis* Christ, Bull. Soc. Bot. France, Mém. 1:44, from Szechuan and Hupeh, is just our species. Shensi: Tai-pei-shan, PURDOM nos. 1, 8, 9, 12.—Recently I have received from Professor BOWER, of Glasgow, a very similar exindusiate form from Darjeeling, Sikkim, British Himalaya, leg. W. CAVE.

POLYPODIUM CLATHRATUM Clarke in Trans. Linn. Soc. II. Bot. 1:559. pl. 82. fig. 1. 1880.—Tai-pei-shan, no. 96.

The young sori are perfectly concealed by large, appressed, thin, peltate scales, which in the mature sori appear as a lacerated, blackish network between the sporangia. This network (clathrate scales) is formed from the thin, peltate scales by the outer (surface) cell-walls having been dissolved, while the remaining cross-walls become thick and black.

*POLYPODIUM EILOPHYLLUM* Diels.—*P. Lewisii* Christ, Nuov. Giorn. Bot. Ital. N.S. 4:97. *pl. 1. fig. 1* (non BAKER).—No. 90.

*Polystichum gracilipes* sp. nov.—Species parva e grege *P. dellodon* (Bak.) Diels rhizomate erecto, squamis angustis fulvis sparse vestito. Stipitibus fasciculatis, gracilibus, ad 6 cm. longis, vix 1 mm. crassis, stramineis, versus basin squamis parvis ovato-acuminatis breviter fimbriatis onustis, sursum rotundis. Lamina lanceolata, simpliciter pinnata, 10–15 cm. longa, vix 2 cm. lata, subtus pallide viridi, subcoriacea, rachi tenui, squamis parvis ovato-lanceolatis longe acuminatis fimbriatis deciduis praedita. Pinnis 20–25-jugis, approximatis, brevissime petiolulatis, quadrangularibus, 8–10 mm. longis, 4–5 mm. latis, basi posteriore cuneata anteriore auriculata, marginibus dentatis, dentibus cuspidatis; pinnis inferioribus parum abbreviatis; pagina inferiore squamis minutis sparse oblecta. Sori inter costam et marginem medium tenentibus vel saepe costae magis approximatis; indusiis magnis, persistentibus, glabris, subintegris.

Shensi: Tai-pei-shan, PURDOM nos. 17 and 31. Closely related to *P. lanceolatum* (Bak.) Diels, from which it differs in its longer stipe, scaly rachis, auricled upper base of the pinnae, and large, persistent sori. Both edges of the pinnae bear about 5 cuspidate teeth each.

*WOODSIA LANOSA* Hook. var. *attenuata*, var. nov.—A typo differt: lamina versus basin sensim attenuatis; pinnis inferioribus minutis; stipitibus vix 2 cm. longa, lamina 5–6 cm. longa, 1 cm. lata; pinnis oblongis nec ovatis.

Shensi: Tai-pei-shan, PURDOM no. 19. Perhaps a new species. The whole frond is very densely covered with long grayish hairs, those of the rachis and costae beneath intermixed with some very thin, narrow, hyaline scales. The rather thick and short rhizome is densely clothed with broad, red scales.



## BRIEFER ARTICLES

### IMBEDDING AND WARMING STAND

(WITH TWO FIGURES)

The warming and imbedding stands for paraffin work used in laboratories are unsatisfactory. Commonly a plate of copper or other metal supported on legs is employed, and by some the apparatus devised by Dr. FERGUSON is used. The apparatus here described was devised five years ago, and it is at the suggestion of various botanists that this description is published. The apparatus is a modification of a familiar temperature stage for microscopes.

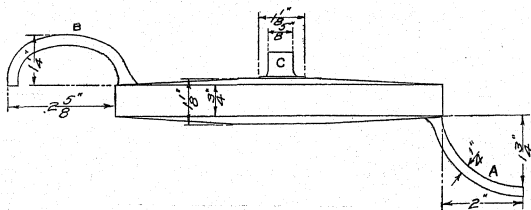


FIG. 1.—Side view of apparatus: A, water intake; B, water outlet; C, opening for thermometer.

The imbedding or warming stages commonly employed are unsatisfactory for the following reasons: (1) temperature cannot be controlled; (2) the imbedding trays cannot be removed quickly for cooling for fear of disturbing the orientation of the imbedded materials (and rapid cooling is essential for best results). The apparatus here described does not possess these objectionable features and permits of rapid work.

The apparatus consists of a copper box constructed on the principle of a water jacket of a condenser. The dimensions of the one employed here are length 20 cm., width 14 cm., and depth 2.8 cm. The box is provided with an inlet for water on the bottom (figs. 1 and 2, A), and an outlet for water on the upper side (figs. 1 and 2, B). There is also provided on the upper side an opening into which is fitted a stopper with a

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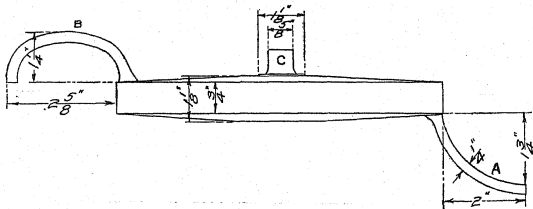


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thermometer (figs. 1 and 2, C). The box is provided with a specially constructed stand, though if desired this may be replaced by a simple tripod stand. Greater stability is secured, however, by having a stand made especially for the copper box.

In constructing the water box it is desirable to have the top of the box made of slightly heavier sheet copper than the bottom, 14 oz. copper for the bottom and 16 oz. copper for the top being satisfactory. By observing this precaution, all bulging due to water expansion or water pressure will manifest itself on the lower side, and a plane surface is maintained on the upper side, which of course is essential for good imbedding.

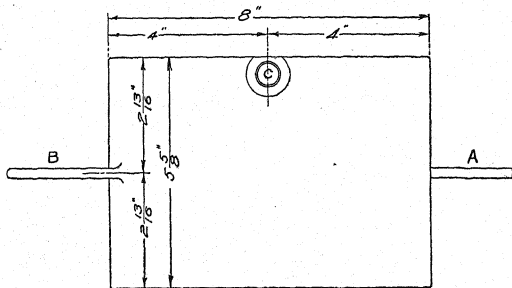


FIG. 2.—Top view of apparatus

In using the apparatus the box is first filled with water and the water supply then shut off. The box is heated by a Bunsen flame, and when the desired temperature is attained, as indicated by the thermometer, the flame may be removed. A constant temperature may also be maintained by continuous heating and then regulating the flow of water; this latter being the better method.

After the material has been oriented, it is rapidly cooled by replacing the warm water with a stream of cold water. If paper trays have been used for imbedding, they may be sufficiently cooled for removal and immersion in water in 30 seconds. For bringing the stand to the proper temperature the same time is required. The top of the box should of course be kept free of paraffin.—L. KNUDSON, *Cornell University, Ithaca, New York.*

## CURRENT LITERATURE

### BOOK REVIEWS

#### Oxidations and reductions

DAKIN's monograph<sup>2</sup> on oxidations and reductions in the animal body is of interest to the plant as well as animal physiologist. Chap. i includes an introduction and discussion of the nature of oxidizing and reducing agents in the body as well as the methods of investigation. Chap. ii deals with the oxidation of saturated fatty acids, unsaturated acids, fatty acids with branched chains, and dibasic acids. Chap. iii discusses the oxidations of  $\alpha$  amino,  $\alpha$  hydroxy, and  $\alpha$  ketonic acids, the oxidation of phenylalanine, tyrosin, tryptophane, and related substances, and the oxidation and reduction of amino acids by microorganisms. Chap. iv treats of the oxidation of carbohydrates; chap. v of the oxidation of purin derivatives; and chap. vi of the oxidation of hydrocarbons, phenols, aldehydes, amines, and indol derivatives. The volume also includes a bibliography of 21 pages and a full index.

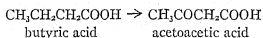
A quotation from the preface gives the point of view of the work: "The statement that fats and sugars are oxidized in the body to carbon dioxide and water, while proteins yield urea in addition, are no longer considered all-sufficient explanations of the chemical rôle of these in animal economy. The study of chemical structure is rapidly changing the whole aspect of biological science, and we may confidently look forward to the time when the orderly succession of chemical reactions constituting the activities of the living cell will be resolved into their individual phases." The author does not enter into "the purely biological aspects of the subject, and also the thermodynamics of the problem of oxidations and reductions have been necessarily omitted as outside the scope of the work. References to many enzymes, oxygenases, peroxidases, etc., which, so far as is known, are without action upon the principal groups of substances which furnish energy to the organism, have also been omitted."

In discussing the nature of the oxidizing and reducing agents of the animal body, he treats as improbable the SCHONBERN theory of "activation of oxygen in its polymerization," the CLAUSIUS and VAN'T HOFF view of the separation of the oxygen into its atoms or ions, and the HOPPE-SEYLER conception of the resolution of the oxygen by nascent hydrogen or other reducing agents. He accepts as very probable the MORITZ TRAUBE peroxide theory of oxidation.

<sup>2</sup>DAKIN, H. D., *Oxidations and reductions in the animal body*. pp. viii+135. New York: Longmans, Green & Co. 1912. One of the monographs on biochemistry.

The main evidence for the theory is the likeness between the oxidations produced in the animal body and those produced in vitro by hydrogen peroxide. It is held that hydrogen peroxide cannot be the effective peroxide in the organism because of the general presence of catalase in active tissues. Organic peroxides are probably the effective agents. The following are listed as peroxide-forming substances: elementary metals and non-metals (hydrogen, phosphorus, zinc, etc.), hydrocarbons, terpenes, alcohols, aldehydes, acids, carbohydrates, ethers, phenols, and aromatic bases and alkaloids.

The following are a few of the reactions mentioned that show the resemblance between the oxidations in the animal body and those carried on in vitro by hydrogen peroxide. The normal fatty acids in the body undergo oxidations in the  $\beta$  position, butyric acid yielding acetoacetic acid. Hydrogen peroxide alone of all the various chemical oxidizing agents brings about precisely the same reaction.



Glucose may be oxidized to glucuronic acid in the body, while hydrogen peroxide is the only reagent capable of effecting this change outside the body. Benzene is oxidized in the body to phenol, catechol, and quinol, and precisely the same reaction is brought about by hydrogen peroxide, but by scarcely any other reagent. Among the dibasic acids, oxalic acid is oxidized readily by most laboratory reagents, but malonic, succinic, and glutaric less readily. In the body oxalic acid is oxidized with great difficulty, but the other three mentioned above very easily. The salts of these dibasic acids act similarly toward hydrogen peroxide. The saturated and unsaturated fatty acids are about equally readily oxidized in the body and by hydrogen peroxide. To most laboratory reagents the saturated acids are far more resistant than the unsaturated. It seems probable from this that botanists<sup>2</sup> are wrong in assuming the greater ease of oxidation of unsaturated acids in the plant.

We need not enter into our knowledge of the details in the course of the complete oxidation of any of the various substances yielding energy. It will suffice to say that many steps are not yet known and others are in dispute, but it is evident that the course of oxidation of any one substance varies considerably with the conditions. The following quotation emphasizes a point commonly stated by botanists: "The specific character of animal oxidations is most remarkable, especially when phenomena such as those presented by diabetes and alcaptonuria are concerned. In these conditions oxidations of a single readily oxidizable product of metabolism (glucose and homogentisic acid) may be completely restrained without in the least impairing the capacity of the body for effecting the oxidation of other substances.—WILLIAM CROCKER.

<sup>2</sup> Bot. Gaz. 54:543-545. 1912.

**Britton and Brown's Illustrated flora**

This well known work, issued in 1896, has reached a second edition.<sup>3</sup> About 300 pages have been added to the text, and the number of species has been increased from 4162 to 4666. Every species is illustrated, and one sees at a glance that the illustrations have been made more effective. This appeal to the eye in distinguishing species is a great contribution to students of the flora represented. It is not merely a service to amateurs, but the professional botanist who is not a taxonomist, but who must know his material, finds his labor of determination vastly lightened. Even for the professional taxonomist, the admirable drawings clear up the vagueness that belongs to many descriptions. In preparing the new edition, good service has been done also in testing out the numerous "new species" that have been described since the first edition. The enthusiasm for new species has been recognized, and the sober second thought has been applied. Some have been relegated to synonymy; others have been noted for further study; and of course some have been accepted.

The area included is probably well known, but a statement of it may be helpful. It extends from the Atlantic Ocean westward to the 102d Meridian, so as to include the whole of Kansas; and northward from the parallel of the southern boundary of Virginia and Kentucky to the northern limits of Labrador and Manitoba. The western limit is crossed only to include the whole of Nebraska.

The nomenclature follows the code recommended by a commission of the Botanical Club of the American Association in 1907, and therefore is somewhat at variance with the Vienna Code of 1905. A unique and extremely valuable feature of the work is the citation of the type species of each genus. So far as the reviewer knows, this has never been done on so extensive a scale, and it must have meant an enormous amount of labor.

The authors are to be congratulated upon this fresh and very helpful contribution to the botanists of the country. Probably not all can own these three large volumes who can own a manual, but they should be so distributed in libraries as to be accessible to all who wish to name plants within the area included.—J. M. C.

**A plant chemistry**

Plant physiologists will welcome the appearance of HAAS and HILL'S<sup>4</sup> treatise on the chemistry of plant products. Section I (49 pp.) is devoted to

<sup>3</sup> BRITTON, N. L., and BROWN, A. An illustrated flora of the Northern United States, Canada, and the British Possessions. Second edition, revised and enlarged. In three volumes: Vol. I (Ophioglossaceae to Polygonaceae), pp. xxi+680; Vol. II, (Amaranthaceae to Loganiaceae), pp. iv+735; Vol. III (Gentianaceae to Compositae), pp. 637. New York: Charles Scribner's Sons. 1913.

<sup>4</sup> HAAS, PAUL, and HILL, T. G. An introduction to the chemistry of plant products. 8vo. pp. 401. London: Longmans, Green & Co. 1913.

fats, oils, waxes, and phosphatides; II (116 pp.) to carbohydrates; III (23 pp.) to glucosides; IV (41 pp.) to tannins; V (30 pp.) to pigments; VI (19 pp.) to nitrogen bases; VII (17 pp.) to colloids; VIII (42 pp.) to proteins; and IX (68 pp.) to enzymes. The book is especially written for plant physiologists, and apparently gives the several subjects their proper proportional consideration as demanded by the aim. It is a very simple, direct statement of the cardinal facts of the subject, giving the main methods, chemical and microchemical, used in the field. The avoidance of a technical form of presentation makes the work usable by those of slight chemical training. In discussing chlorophyll, the authors make the barest mention of the older work on the subject, done, as they say, in the main with impure products. The discussion is based on the late work of WILLSTÄTTER and his students, and of TSWERT. This gives in the simplest and most direct way the picture of our present knowledge of chlorophyll. The treatment of chlorophyll is typical of the method of the book and shows one of its great virtues. No mention is made of the important work of IWANOW on metabolism of fats, but this could hardly be expected, since the book deals with little literature of a later date than 1910. The treatise is one that every plant physiologist and probably every botanist dealing at all with the physiology of plants will want on his desk.—WILLIAM CROCKER.

#### MINOR NOTICES

**Nigerian plants.**—The British Museum has published<sup>5</sup> a catalogue of the plants of the Oban District of South Nigeria collected by Mr. and Mrs. P. AMAURY TALBOT during 1909 to 1912. The determinations have been made by several specialists, and the collection has proved to be unusually rich in novelties. Of the 1016 species and varieties enumerated, 195 are new, and among them are 9 new genera, as follows: *Alphonseopsis* and *Dennettia* (Anonaceae), *Crateranthus* (Myrtaceae), *Afrohamelia*, *Dorothea*, *Diplosporopsis*, and *Globulostylis* (Rubiaceae), *Scyphostrychnos* (Loganiaceae), *Talbotia* (Acanthaceae), and *Amauriella* (Araceae). The new species are distributed among 31 families, those receiving the largest additions being Rubiaceae (34), Acanthaceae (21), Orchidaceae (20), and Apocynaceae (12).—J. M. C.

#### NOTES FOR STUDENTS

**Caprification.**—BAKER<sup>6</sup> has published an interesting study of caprification in a Philippine *Ficus*. On some trees of *Ficus nota* there are produced pear-shaped inflorescences which when mature contain gall flowers and staminate

<sup>5</sup> RENDLE, A. B., BAKER, E. G., WERNHAM, H. F., and MOORE, S., Catalogue of the plants collected by Mr. and Mrs. P. A. TALBOT in the Oban District, South Nigeria. pp. x+157. pls. 17. London: Longmans, Green & Co. 1913.

<sup>6</sup> BAKER, C. F., A study of caprification in *Ficus nota*. Philippine Jour. Sci. 8: Section of Gen. Biol. 63-83. 1913.



flowers. On other trees of the same species only carpellate flowers are found within the inflorescence, although in the latter cases rudiments of stamens sometimes appear. Pollination is effected by means of a new species of *Blastophaga* (*B. nota* Baker), which in its appearance and behavior presents striking differences from the published accounts of the pollination of the fig.

Upon gall-bearing trees of *Ficus nota*, the production and maturing of inflorescences is almost continuous, and the broods of the gall-producing *Blastophaga* constantly overlap one another, thus failing to show the definite seasonal stages of insect and gall as described in the case of the Smyrna fig. A short time before the opening of the staminate flowers in the gall-fig or caprifig, the wingless males of *Blastophaga* emerge from some of the galls within the inflorescence. These males immediately begin gnawing holes into other galls within the same inflorescence, deserting these holes at once when they are found to contain insects other than the females of *Blastophaga*, and copulating with the females when they are present. After copulation the male does not enlarge the opening, thus assisting the female to escape from the gall as has been stated in other descriptions, but it proceeds to gnaw openings into other galls which may or may not contain females of *Blastophaga*. Most of the males die soon after copulation with one or a few females. The females gnaw their way out of the galls which inclose them. The interior of an inflorescence soon becomes an active mass of winged females of *Blastophaga*, and of insects of several other genera which mature within the gall at the same time. The stamens mature at this time and dehisce naturally (not cut open by the male *Blastophaga* as described for other species of *Ficus*), and the bodies of the females become dusted with pollen. The scales which have kept the inflorescence closed against the escape of the insects up to this time now wither and the females escape. Some of these females fly to younger inflorescences upon the same tree or upon similar gall-bearing trees and enter the inflorescences. In the young gall-forming inflorescences the carpels are of such form that the insect can insert the ovipositor into the funnel-shaped stigma and place the egg within the ovary of the carpel. When this has been done the conditions are supplied for the development of new galls. But when these females fly to trees upon which are the inflorescences which produce ripened figs, the story is different. In the inflorescences of these trees the styles and stigmas are of such form that the insects cannot place their eggs within the ovary, and the females run about within the inflorescence, finally dying without having placed their eggs within the ovaries of the flowers. But meantime, as they have moved about within the inflorescence the insects have placed upon the stigmas the pollen which they brought from the gall figs or caprifigs. Fertilization, seed formation, and ripening of the figs follow.

BAKER finds not only this new species of *Blastophaga*, but one new genus and five new species of parasitic insects accompanying *Blastophaga* in infesting the inflorescences of *Ficus nota*.—O. W. CALDWELL.

**Anatomy of Japanese conifers.**—The difficulty in distinguishing the wood of closely related conifers by their anatomical structure is clearly illustrated by FUJIOKA's detailed study<sup>7</sup> of the anatomy of 37 species of Japanese Coniferales. The primary object of the investigation, as outlined in the preface, was to secure a more reliable basis for distinguishing the various Japanese woods of similar external appearance. In the "Tabelle zum Bestimmen" which summarizes the results of the investigation, the 19 genera investigated are separated into 16 groups. Evidently no simple and reliable basis for distinguishing species was discovered, nor were the following genera separated: *Taxus* and *Torreya*; *Thuyopsis*, *Cryptomeria*, *Chamaecyparis*, and *Cunninghamia*. The diagnostic characters used in the classification are those used by GOTHAN<sup>8</sup> in his key to the wood of the gymnosperms and are therefore subject to similar criticisms.

The use of traumatic resin canals as a basis for separating the Abietae (*Abies* and *Tsuga*) from other conifers is unreliable, since any given specimen submitted for identification may be uninjured and therefore may not possess these structures. As the reviewer has pointed out,<sup>9</sup> tertiary thickenings are not invariably a reliable diagnostic character in separating the wood of *Larix*, *Pseudotsuga*, and *Picea*. Similarly, variations in ray parenchyma pitting are of doubtful value in distinguishing the wood of the various genera of the Cupressineae. That the cupressineous type of ray pitting is a reduction from the abietineous type ("Abietineen Tüpfelung") is clearly shown by the persistence of the latter in the Taxodineae and Cupressineae in regions of phylogenetic significance, for example, cone axis, injured wood, young root, etc. As is commonly the case with structures undergoing reduction, the ray pitting is extremely variable in a given species or genus of the Cupressineae, just as is the occurrence of marginal tracheids and the recurrence of resin canals in the wood of the Abietae and *Sequoia*.

The study of many gymnosperms and angiosperms emphasizes the fact that although internal structures are invaluable in blocking out the general outlines of a natural classification of plants, they are too conservative to be significant in distinguishing closely allied species and genera.—IRVING W. BAILEY.

**Cecidology.**—Among the European contributions is a paper by SCHELLENBERG<sup>10</sup> in which the author claims that galls caused by fungi serve for storage for

<sup>7</sup> FUJIOKA, M., Studien über den anatomischen Bau des Holzes japanischen Nadelbäume. Jour. Coll. Agric. 4:201-236. pls. 18-24. 1913.

<sup>8</sup> GOTHAN, W., Zur Anatomie lebender und fossiler Gymnospermen-Hölzer. Abh. Preuss. Geol. Landesanstalt. Berlin. 1905.

<sup>9</sup> BAILEY, I. W., The structure of the wood in the Pineae. BOT. GAZ. 48:47-55. pl. 5. 1909.

<sup>10</sup> SCHELLENBERG, H. C., Über Speicherung von Reservestoffen in Pilzgallen. Verhandl. Schweiz. Naturl. Gesells. 94:277-279. 1911.

the parasite. These storage materials are the same as those found in other parts of the plants except that they have a much higher concentration.

SWANTON<sup>11</sup> describes a mite gall on *Geranium lucidum* caused by *Eriophyes geranii canestrini*. This gall does not occur on other species of *Geranium* on the British Islands, but does occur on three other species on the continent. *E. rubiae* Can. attacks the apical leaves of *Rubia peregrina*, causing them to appear as flowers.

In the American literature we note a new species by COCKERELL<sup>12</sup> under the name of *Cecidomyia peritomatis*. This is especially interesting because of the very few galls known on Capparidaceae.

A very interesting paper by WHITE<sup>13</sup> on the bearing of teratological development in *Nicotiana* on the theories of heredity begins with a brief review of our knowledge of teratology. The mutant of *Nicotiana tabacum* was obtained from Alquiza, Cuba, in 1907. The malformation consisted of a flattened stem accompanied by many smaller teratological features, especially in the flowers. Five generations, involving more than 1000 plants, have been grown, each individual showing the original mutant characters which are shown in tables. The results of his experimental work are summarized as follows: "From the results of hybridization and selection, one may draw the conclusion that the fasciated mutant differed from the normal parent strain by only one factor, and that it represents a mutation upon the variability of which selection has no modifying effect. The character appears to be due to the one underlying cause, and its variableness is only the external manifestation of the capricious working of that cause." The author also gives a very interesting and suggestive discussion of the cytology of the mutant and the normal, which he concludes by saying that "the evidence warrants one in the suggestion that chromosomes are characters of the zygote and gametophyte, on the same development with other plant characters."—MEL. T. COOK.

**Araucarineae.**—THOMSON<sup>14</sup> has made a detailed study of the anatomy of the araucarians, and has reached certain conclusions in reference to the affinities of this much discussed group. He has taken into account leaf gaps, leaves, pitting of secondary tracheids (including bars of Sanio), resin tissue, medullary rays, bast and periderm, annual ring and tangential pitting, and fossil forms. The recent discussion concerning the origin of the araucarians has presented

<sup>11</sup> SWANTON, E. W., New and rare British plant galls. Jour. Botany 50:283, 284. 1912.

<sup>12</sup> COCKERELL, T. D. A., A new gall on *Peritoma serrulatum*. Jour. Econ. Entomol. 6:279, 280. 1913.

<sup>13</sup> WHITE, O. E., The bearing of teratological development in *Nicotiana* on the theories of heredity. Amer. Nat. 47:206-228. 1913.

<sup>14</sup> THOMSON, ROBERT BOYD, On the comparative anatomy and affinities of the Araucarineae. Phil. Trans. Roy. Soc. London B 204:1-50. pls. 1-7. 1913.

two alternatives: derivation from the lycopods, suggested by SEWARD; or derivation from the Abietineae, supported by JEFFREY. THOMSON dissents from both, and concludes that the araucarians have been derived directly from the Cordaitales. The objection to a lycopod origin is based chiefly upon the presence of leaf gaps, which THOMSON regards as of fundamental importance in indicating an origin by way of the fern stock.

The objections to derivation from the Abietineae deal with many details, the pith of them being that in the various anatomical details used araucarians resemble Cordaitales more than they do Abietineae. JEFFREY has appealed to the Mesozoic plexus of "Abietinean-Araucarian" forms as indicating the origin of araucarians from the Abietineae; but THOMSON concludes that these transition forms indicate that Abietineae have been derived from the araucarians, and he claims that this conclusion is confirmed by the fact that the araucarians are of greater geological age than the Abietineae. This last statement is based upon the fact that THOMSON and ALLIN<sup>15</sup> investigated certain Permian and Carboniferous forms that had been referred to *Pityoxylon* and found that they do not belong to Abietineae.—J. M. C.

**Marine flora of Woods Hole.**—For a number of years DAVIS has been studying the marine flora of Woods Hole and vicinity, part of the time in connection with the biological survey of the Bureau of Fisheries. The results have now appeared in two sections<sup>16</sup> of a bulletin of the Bureau of Fisheries, and represent the most complete study of our marine algal flora up to this time. The first section deals with the ecology of the flora, such factors being discussed as the coast, the bottom in deeper water, the tides and tidal currents, the effect of ice, depth of water, light, temperature and seasonal changes, and salinity of the water. The characteristic algal associations are described, their number reaching 57. Special reports are also made on the algae of Spindle Rocks, Woods Hole Harbor, and on the distribution of the marine algae in the deeper waters of Buzzards Bay and Vineyard Sound. This section is accompanied by 47 descriptive charts.

The second section is a catalogue of the marine flora, the number of forms enumerated, with data concerning their distribution, being as follows: Cyanophyceae 37, Chlorophyceae 52, Phaeophyceae 74, Rhodophyceae 96.—J. M. C.

<sup>15</sup> THOMSON, R. B., and ALLIN, A. E., Do the Abietineae extend to the Carboniferous? BOT. GAZ. 53:339-344. 1912.

<sup>16</sup> DAVIS, BRADLEY MOORE, General characteristics of the algal vegetation of Buzzards Bay and Vineyard Sound in the vicinity of Woods Hole; also A catalogue of the marine flora of Woods Hole and vicinity. Bull. Bur. Fisheries 31:443-544, 795-833. 1911.

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TEMPERATURE COEFFICIENTS IN PLANT GEOGRAPHY  
AND CLIMATOLOGY<sup>1</sup>

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AND

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(WITH THREE FIGURES)

## Introduction

Plant geographers and climatologists have long been convinced that temperature is one of the most important of the conditions governing the distribution of plants and animals, but very little has as yet been accomplished toward finding out what sort of quantitative relations may exist between the nature of floral and faunal associations and the temperature conditions that are geographically concomitant therewith. The progress of descriptive ecology has shown clearly enough that these associations have their geographical limits, and increased accuracy of description has developed hand in hand with the idea that association boundaries must be considered as peripheries of certain complexes of environmental conditions. It is common, in recent papers upon plant and animal geography, to give considerable attention to descriptions of the climatic complexes which characterize the vegetational or faunal areas dealt with, but such description of climates has thus far usually consisted in the mere quotation or compilation of various meteorological data. In order to correlate these data with physiological phenomena, in such a way as to throw light upon the effective climatic

<sup>1</sup> Botanical contribution from the Johns Hopkins University, no. 36.

characteristics of the areas occupied by the respective associations, it will first be necessary to devise methods, and frequently instruments also, by which the climatic conditions of any given area may be so integrated as to bring out their relations to the processes of growth and reproduction in organisms. In the beginning, such methods will usually need to deal with single conditions or factors as they influence physiological phenomena, but biological science must eventually appreciate that single conditions, or any group of conditions comprising less than all the effective ones, cannot be considered as determining natural processes. Complexes of environmental factors must needs be analyzed into simpler ones, for purposes of study, but after knowledge of the effectiveness of each has been experimentally obtained (through maintaining all conditions other than the one in question not only constant but at a known and stated value or intensity), they must then be recombined and their effects integrated before real knowledge may be available.

If a plant thrives in a given locality, so as to form a part of the flora of that locality, it is obvious that there have not occurred here, since the arrival of its ancestors, any environmental conditions incompatible with its existence. In this we merely make the biological observation that the organism is and has been *adapted*<sup>2</sup> to the environment in which we find it, and that the environment is and has been *adapted* to the organism. Thus, when the range or geographical extent of an organism or association has been determined, it is clear that the area or areas so circumscribed have been characterized for some time in the immediate past by conditions none of which have been adverse enough to annihilate the forms dealt with. Outside of these areas the conditions have not been such as to bring about the permanently successful entrance of these forms, up to the present time. It is of course clear that entrance may have been accomplished here, but if this has been the case each such entrance must have been followed by the occurrence of annihilating conditions.

<sup>2</sup> On a somewhat matter-of-fact view of biological adaptation, see the following paper: LIVINGSTON, B. E., Adaptation in the living and non-living. *Amer. Nat.* 47:72-82. 1913. See also HENDERSON, L. J., The fitness of the environment. New York. 1913.

Furthermore, the internal conditions (or the nature) of an organism being duly considered, the success attained by it in any locality is an index of the extent to which the environmental conditions of that place have been favorable to successful growth, both vegetative and reproductive. Thus it frequently becomes apparent that the conditions of one locality have been more or less favorable to the success of a given form than have those of another locality.

The difficulty, however, which ecological science has not yet been able to surmount lies in the extreme complexity of the system of conditions which affect the success of organisms. The numerous component factors of an environmental complex fall into categories of *water, non-aqueous materials, heat, light, and mechanical conditions*. It is obvious that the limiting condition that has prevented the occurrence of a certain plant, for example, in a given area, may belong to any one of the foregoing categories; mechanical conditions may have failed to bring the seed hither, moisture conditions, or other conditions of material supply or removal, may have annihilated the plant after it was really introduced, or annihilation may have followed from adverse temperature or light conditions, etc.

The distributional problem is made still more complicated and difficult by the perfectly clear but seldom emphasized proposition, that each separate or component condition of an environmental complex is a variable in at least two dimensions, as it were; each such condition must always be considered with regard to its *intensity* and also with regard to its *duration*. To illustrate by means of a water factor, a desiccated soil may cause the death of a given seed, but this result would probably not be accomplished if the soil were dry for only a day or a month; the condition of dry substratum must be maintained for a certain minimum period if it is to be effective as a limiting condition. Some less well defined environmental factors have to be considered in regard to a third dimension which may vary, namely, *quality*. Light, for example, may reach chlorophyll tissue in seemingly sufficient intensity and during an apparently sufficient time period to produce a requisite amount of carbohydrate, but this may fail to happen because the radiant energy is of too short or too long wave-length. Here quality is as important as is either intensity or duration.

Finally, the difficulties of distributional, as of other physiological problems, are greatly aggravated by the fact that each organism is always in process of internal alteration, passing thus through various more or less well defined and conveniently separable phases of development. Thus, a certain quality, intensity, and duration of the light condition may be wholly without sensible effect upon an unsoaked seed, while the same dimensions of the same factor may be fatal to the same plant when in a more active developmental phase. In the study of external conditions it is thus useless to attempt to establish relations between these and organisms, unless adequate consideration is given to internal conditions or developmental phases of the latter. Quantitative ecology must not only try to find out to what environmental conditions its organisms are subjected, and to what degree and for how long these conditions are present, but it must also determine during what developmental phases of the organisms the conditions are effective. It is this consideration which makes it quite impossible to establish ecological relations without adequate knowledge of the physiological nature, at different developmental stages, of the forms dealt with.

Leaving temporarily out of account those mechanical conditions which may, in the past, have acted to transport plants from one geographical area to another, we are aware that the environmental conditions which control growth and reproduction, after the introduction of an organism into a given region, are nearly always naturally in a state of flux. Day and night changes and the march of the seasons, together with more markedly irregular fluctuations in wind, precipitation, evaporation, etc., all produce their effects upon both living things and non-living ones, but these effects are usually much more pronounced in the case of organisms than in that of inanimate objects. As has been previously emphasized by one of the writers,<sup>3</sup> an organism furnishes, at any instant, a summation of the effects of all the processes which have gone on in the body during its previous developmental history. One of our greatest difficulties, however, lies in the fact that we are totally unable to differentiate this integrated record, but we may nevertheless be

<sup>3</sup> LIVINGSTON, B. E., Climatic areas of the United States as related to plant growth. *Proc. Amer. Phil. Soc.* 52: 257-275. 1913.



sure that each of its component terms involves at least a quality, intensity, and duration of some environmental factor. It is thus seen that what is most needed for etiological studies of the geographic distribution of organisms, as well as for all other studies which deal with the conditioning or causation of life processes, is comparative measurements of these processes, or of groups of them, and corresponding measurements of the conditioning environmental factors. To accomplish such measurements and thus to institute the requisite comparisons is now practically impossible in most cases; methods need first to be devised and then to be applied, and the world does not yet offer satisfactory facilities for either. Measurements which can be carried out in present-day laboratories, while applicable in some cases, especially with forms not influenced by light, are quite inadequate even for making a rational beginning in the quantitative study of environments in general. In planning a campaign for this sort of study it is obvious that the effects produced by any environmental factor cannot be adequately studied unless that factor, as well as all the other effective ones, is under control by the experimenter. A laboratory where such experimentation as is here indicated may be carried out is now feasible, with recent advances made in the physical sciences and in physiology, and the scientific and practical importance of the results to be obtained, especially from the standpoint of agriculture and forestry, may perhaps soon warrant the provision of the needed facilities, somewhere in the world.<sup>4</sup> Until such facilities may have become available, it will be profitable, however, to prepare the way for them by carrying out such quantitative or semi-quantitative comparisons between vital activities and environmental conditions as are at present possible.

The present paper involves some of the results of an attempt to find a rational method for interpreting climatic temperature data for phytogeographic purposes. This sort of study is somewhat simplified, in the case of plants, by the fact that the temperature of

<sup>4</sup> This need was strongly emphasized over two decades ago by ABBE, who also quotes DECANDOLLE to the same effect. See ABBE, C., First report on the relations between climates and crops. U.S. Dept. Agric., Weather Bureau, Bull. 36. p. 23, 1905.

the plant body follows very closely upon that of the surroundings, and that soil temperature and air temperature are, roughly speaking, somewhat closely related. Furthermore, the heat condition of plants, as approximately measured by the temperature of the surrounding air, varies only in respect to intensity and duration; qualitative fluctuations are not met with here. Geographically, the present study deals with the area occupied by the United States.

### Direct temperature summation

The effectiveness of temperature conditions to promote plant growth in any locality has been measured by phenologists,<sup>5</sup> by means of the direct summation of the daily mean temperatures, such summation extending through the period of any particular phase of plant development which might be considered; for example, the period extending from the time of germination to that of flowering or of seed-maturation. In these temperature summations a certain minimum temperature is assumed as a starting-point, and the amount added to the summation for each day as the season advances is the number of degrees, above the assumed minimum, which represents the mean temperature for that day. The minimum has sometimes been 0° C., more often a somewhat higher temperature. In the employment of such temperature summations, each station of observation is characterized each year by its summation index, and after a period of years these indices may be averaged to give a measure of the temperature factor in general, for that particular place.

In a way somewhat similar to that followed by workers in phenology, MERRIAM<sup>6</sup> has obtained normal summation indices of temperature for a large number of stations in the United States, and has presented these in the form of a chart showing climatic zones, each characterized by its own range of temperature summa-

<sup>5</sup> In this connection see ABBE, *loc. cit.*

<sup>6</sup> MERRIAM, C. H., Laws of temperature control of the geographic distribution of animals and plants. *Nat. Geog. Mag.* 6:229-238. 1894. The same work was again reported, in still more abbreviated form, in part III of the following paper: MERRIAM, C. H., Life zones and crop zones of the United States. U.S. Dept. Agric., Div. Biol. Survey, Bull. 10. 1898.

tion indices. So far as we know, this is the first chart of its kind to be prepared upon the basis of temperature summation, and the temperature zones of this writer have come into rather general use among American phytogeographers.

A method of direct summation, similar to those employed by phenologists but dealing in a more refined way with the temperature conditions of plant environments, has been described by MACDOUGAL.<sup>7</sup> This author summed the temperatures above the freezing-point of water for the period occupied by certain developmental phases of certain plants; but, instead of adopting as the terms of the summation the daily means or the means of daily maxima and minima, his integration was performed, with a planimeter, upon automatically traced thermograph records. The resulting indices of environmental heat conditions are expressed by MACDOUGAL in terms of "hour-centigrade-degree" units. This method has never been employed in climatological or phytogeographical studies, so far as we are aware. It seems to be the simplest and most promising of all the direct summation methods, but of course requires reliable thermograph tracings. Each year or season is to be treated separately, and the resulting annual indices may be averaged for a period of years, to give a normal index.

### Temperature efficiencies

Although such temperature summations as those of the phenologists and of MERRIAM have seemed in many instances to furnish data consistent among themselves and constituting on an empirical basis an apparently reliable criterion for the measurement of the intensity and duration aspects of the temperature factor, yet it must be regarded as highly improbable that any fundamental and general principle regarding the influence of temperature on plant life may be derived from the relations thus brought out. It seemed to us that the apparent value of temperature summations must rest upon some basic principle of physiology not indicated in the summations themselves.

<sup>7</sup> MACDOUGAL, D.T., The temperature of the soil. Jour. N.Y. Bot. Garden 3:125-131. 1902.

Now, as far as temperature influence is concerned, the most fundamental generalization that physiology has yet been able to attain is that which may be termed the principle of temperature coefficients. This is primarily the application of the chemical principle of VAN'T HOFF and ARRHENIUS to vital phenomena. This principle states that, within limits, the velocity of most chemical reactions doubles or somewhat more than doubles for each rise in temperature of  $10^{\circ}\text{C}$ .<sup>8</sup> The principle fails to hold rigidly, even in the somewhat vague form in which it is stated, but it seems to express in an approximate way a fairly general truth in chemical phenomena.

A considerable literature has developed about the application of this chemical principle in physiology. CLAUSEN<sup>9</sup> determined the rate of evolution of carbon dioxide from seedlings and buds at several different temperatures and found that this rate somewhat more than doubled for each rise in temperature of  $10^{\circ}$ , up to an upper limit of about  $40^{\circ}\text{C}$ . COHEN<sup>10</sup> calculated from measurements of O. HERTWIG<sup>11</sup> that the time consumed by developing frog's eggs, for the completion of certain developmental phases, about doubled for each *fall* in temperature of  $10^{\circ}$ . Miss MATTHAEI<sup>12</sup> studied the influence of temperature on the evolution of carbon dioxide from leaves in darkness and also on the fixation of this gas by leaves in light, and showed that the VAN'T HOFF-ARRHENIUS principle holds also for these plant processes. A temperature coefficient of the same order of magnitude as is required by this principle was found, for a number of different physiological pro-

<sup>8</sup> For one clear statement of this principle see VAN'T HOFF, J. H., Lectures on theoretical and physical chemistry, translated by R. A. LEHFELDT. London. No date (author's preface dated 1898). Part I. pp. 227 f.

<sup>9</sup> CLAUSEN, H., Beiträge zur Kenntnis der Athmung der Gewächse und des pflanzlichen Stoffwechsels. Landw. Jahrb. 19:893-930. 1890.

<sup>10</sup> COHEN, E., Lectures on physical chemistry for physicians and biologists. Translated by MARTIN H. FISHER. New York. 1902.

<sup>11</sup> HERTWIG, O., Über den Einfluss der Temperatur auf die Entwicklung von *Rana fusca* und *Rana esculenta*. Arch. f. Mikroskop. Anat. und Entwicklungsgesch. 51:319-381. 1898.

<sup>12</sup> MATTHAEI, GABRIELLE L. C., Experimental researches on vegetable assimilation and respiration. III. On the effect of temperature on carbon dioxide assimilation. Phil. Trans. Roy. Soc. London B 197:47-105. 1904.

cesses in animals, by LOEB<sup>13</sup> and his co-workers, and SNYDER<sup>14</sup> has emphasized the value of non-chemical temperature coefficients in the study of physiological velocities. An excellent statement of this whole problem, especially with regard to plants, is included in BLACKMAN's Dublin presidential address,<sup>15</sup> where he discusses also the determination of the effect of temperature on the rate of division of the flagellate *Chilomonas paramecium*, as carried out by MALTAUX and MASSART,<sup>16</sup> and points out that the temperature coefficient here really dealt with has a magnitude of about 2.4. BLACKMAN's concluding sentences in this address are worthy of quotation here, for their general bearing on the nature of the question with which we are dealing:

To me it seems impossible to avoid regarding the fundamental processes of anabolism, katabolism, and growth as slow chemical reactions catalytically accelerated by protoplasm and inevitably accelerated by temperature. This soon follows if we once admit that the atoms and molecules concerned possess the same essential properties during their brief sojourn in the living nexus as they do before and after.

In much of the work that has been published on vital temperature coefficients, relatively simple physiological processes have been considered, and it seems allowable to conclude, at least tentatively, that most of the elementary chemical processes of living things go on according to the principle of VAN'T HOFF and

<sup>13</sup> An apparently complete list of citations for the contributions bearing upon this general subject, including those here referred to, up to November 1908, is given in the following personally polemical article: LOEB, J., ROBERTSON, T. B., MAXWELL, S. S., and BURNETT, T. C., On the encouragement of Mr. CHARLES D. SNYDER. *Science* N.S. 28:645-648. 1908. This paper is to be read in connection with SNYDER's calmer reply: SNYDER, C. D., A reply to the communication of Messrs. LOEB, MAXWELL, BURNETT, and ROBERTSON. *Science* N.S. 28:795-797. 1908.

<sup>14</sup> SNYDER, C. D., Der Temperaturkoeffizient der Geschwindigkeit der Nervenleitung. *Arch. Anat. und Physiol., Physiol. Abt. Jahrg.* 1907. 113-145.

———, A comparative study of the temperature coefficients of the velocities of various physiological activities. *Amer. Jour. Physiol.* 22:209-334. 1908.

<sup>15</sup> BLACKMAN, F. F., The metabolism of the plant considered as a catalytic reaction. Presidential Address, Botanical Section, British Association, Dublin meeting, 1908. *Science* N.S. 28:628-636. 1908.

<sup>16</sup> MALTAUX, MARIA, and MASSART, JEAN, Sur les excitans de la division cellulaire. *Ann. Soc. Roy. Sci. Méd. et Nat. Bruxelles* 15:1-53. 1906; *Recueil de l'Inst. Bot. Bruxelles* 4:369-421. 1906.

ARRHENIUS, and that such processes possess temperature coefficients, within the ordinary limits of environmental temperatures, of an order of magnitude of from about 2.0 to about 2.5. This may be regarded as a fundamental principle in physiology.

When, however, many of these elementary or component processes are combined into a complex resultant, such as we have in physiological growth, for example, it is not immediately clear on a priori grounds that temperature coefficients of this same order of magnitude must obtain. RUSSELL<sup>17</sup> states rather authoritatively that "the effect of temperature on the *rate of growth* of a plant is in nowise like its effect in accelerating chemical change," and cites the work of BIALOLOCKI<sup>18</sup> in support of this view. The last named writer studied the influence of temperature upon the rate of growth of barley, and his results appear to show (see RUSSELL's graph, p. 21) that the value of the temperature coefficient in this case alters markedly with the temperature itself. Considering, however, the fact that these results of BIALOLOCKI appear to differ very markedly from those of the later workers who have dealt with the question, we are inclined not to give them such conclusive weight as does RUSSELL. In considering the matter before us, it is to be remembered that the principle of VAN'T HOFF and ARRHENIUS has never been supposed to hold, even for simple chemical reactions, excepting *between certain limits*, and that these limits should not be expected to be the same for all processes. Furthermore, as has been emphasized by BLACKMAN,<sup>19</sup> RUSSELL (*loc. cit.* pp. 20 f.), MITSCHERLICH,<sup>20</sup> and others, the full possible effect of a rise in temperature is frequently precluded by the failure of some other environmental condition correspondingly to alter. To illustrate, we may suppose (as BLACKMAN, *loc. cit.* 1908, suggests) that the

<sup>17</sup> RUSSELL, E. J., Soil conditions and plant growth. London. 1912.

<sup>18</sup> BIALOLOCKI, J., Über den Einfluss der Boden wärme auf die Entwicklung einiger Culturpflanzen. Landw. Versuchsstat. 13:424-472. 1870.

<sup>19</sup> *Loc. cit.*; also BLACKMAN, F. F., Optima and limiting factors. Ann. Bot. 19:283-295. 1905.

<sup>20</sup> MITSCHERLICH, E. A., Das Gesetz des Minimums und das Gesetz des abnehmenden Bodenertrages. Landw. Jahrb. 38:537-552. 1909.

———, Über das Gesetz des Minimums und die sich aus diesem ergebenden Schlussfolgerungen. Landw. Versuchsstat. 75:231-263. 1911.

velocity of a given chemical process doubles with each rise of  $10^{\circ}$  in temperature, but that the process is retarded by the accumulation of the products of the reaction. In such a case it is obvious that, with increasing temperature, a point might sooner or later be reached at which the removal of these products might not proceed rapidly enough to allow the full temperature effect to become manifest. Thus, the rate of removal of the products must be adequately increased with the rise in temperature; otherwise the effect of this rise becomes masked by the effect of another variable, namely the mass action of the products. From these considerations it does not seem surprising that complex vital processes such as growth may frequently fail, under natural conditions, to exhibit the usual chemical temperature coefficient. In some of these cases, proper alterations in other environmental factors might disclose the otherwise obscured coefficient; in other cases the limitations might lie in the nature of the protoplasmic mixture, and the obscuring of the coefficient might persist in spite of any attempt at external adjustment.

The most satisfactory study on the influence of temperature upon growth rates in plants, so far as our knowledge goes, is that of PRICE,<sup>21</sup> who determined temperature coefficients for the opening of flower buds of the plum, peach, apple, and other fruits, and found the VAN'T HOFF-ARRHENIUS principle generally to hold. Beginning with resting buds, the time period required for blooming is reduced about one-half for each rise in temperature of  $10^{\circ}$  C. The same author figures maize seedlings which suggest that the rate of growth in length of shoot about doubles for each rise of  $10^{\circ}$ .

The present aspect of the entire question leads us to the conclusion that there are many cases in which growth rates and other complex processes in plants and animals exhibit temperature coefficients of about 2.0, and that, in other cases, this same coefficient may probably be operative, but may be obscured by the limiting effect of some other environmental or internal condition. It must also be supposed that temperature coefficients of other orders of magnitude may be encountered, not only for complex life

<sup>21</sup> PRICE, H. L., The application of meteorological data in the study of physiological constants. Ann. Rep. Virginia Agric. Exp. Sta. 1909-1910.

processes, but also for some of the elementary ones. The elementary processes of growth itself (that is, the *immediate* phenomena which condition growth, the ones first met with in a rational attempt to analyze the complex process) are all or nearly all physical in nature, and not to be regarded as chemical. They include such physical changes as coagulation, precipitation, alterations in elasticity, swelling by imbibition and osmotic action, and many others. It thus becomes apparent that the reason why the chemical temperature coefficient appears to be manifest in growth phenomena cannot be that these phenomena are primarily and immediately chemical in their nature, but that, physical though they are, they depend in turn upon other internal changes that are unquestionably chemical. Thus, for a single example, the precipitation or coagulation of colloid material met with in the formation of cell walls in plants must logically be dependent upon the continuous presence of the precipitating substances in the peripheral layer of the protoplasm of each growing cell, and within a certain range of concentration, and this continuous presence indicates chemical processes which must be effective not very far back (in the chain of causally connected phenomena) from the precipitation itself. Under such circumstances it might be expected that a physical complex such as growth would frequently exhibit a chemical temperature coefficient.

The fundamental physical changes which make up growth have not yet been studied sufficiently to permit the making of any estimate regarding the orders of magnitude of their temperature coefficients; nevertheless, we are certain that some of these coefficients possess values widely different from that postulated by the VAN'T HOFF-ARRHENIUS principle. Thus, for example, the temperature coefficient of osmotic pressure, within the range encountered in living cells, approximates the familiar quantity 0.03665, as usually employed, for each single degree above the zero point of the Centigrade scale, the pressure at 0° being taken as a basis. If the pressure at 0° be considered as unity, the pressures at 4°, 14°, 24°, and 34° become 1.01466, 1.05131, 1.08796, and 1.12461, respectively, and for each 10° rise in temperature the pressure is increased by only about 0.04.



On the other hand, within the temperature range with which physiology deals, some physical phenomena exhibit temperature coefficients somewhat closely approximating the order of magnitude called for by many chemical reactions. To illustrate, the vapor tension of water at  $4^{\circ}$  is 6.097 mm. of mercury, and the tensions at  $14^{\circ}$ ,  $24^{\circ}$ , and  $34^{\circ}$  are 11.908 mm., 22.184 mm., and 39.565 mm., respectively. Here the three temperature coefficients corresponding to these three rises of  $10^{\circ}$  in temperature are 1.95, 1.86, and 1.78, respectively. The vapor tension of water must be accounted an important condition for all transpiring plants; this pressure may be regarded as the driving force of evaporation, thus constituting the fundamental energy condition of the phenomenon of transpiration.

#### Application of temperature coefficients to climatology

If the processes of growth and development do really exhibit temperature coefficients, it is plain that the study of environmental integrations should deal with these rather than with temperatures directly. It is also plain that if direct temperature summations do, in certain cases, furnish adequate criteria for evaluating the effectiveness of temperature conditions, then this state of affairs must be true only within certain limits, and the experimental study of temperature coefficients furnishes the only adequate means for locating these limits and establishing the direct summations upon a rational basis. It seems worth while, therefore, to make a first attempt in the direction of the application of velocity coefficients to the study of effective temperature conditions as these characterize climatic and vegetational areas. Such an attempt, of course, must be very unsatisfactory from an idealistic point of view; nevertheless, it should serve to emphasize the need of quantitative studies in this connection and should also be of value in showing what sort of climatic and distributional observations are most likely to be of value as ecology becomes more exact.

For the present study we have tentatively assumed that the temperature coefficient of growth and development has a value of 2.0 for each rise of  $10^{\circ}$  C., a value which somewhat closely approxi-

<sup>22</sup> BIEDERMAN, R., *Chemicker Kalendar*. 1903. Bd. 2. Berlin. 1903. pp. 84, 85.

mates those which have so far resulted from most physiological studies of temperature relations. It seems that this value is more likely to be too low than too high for ranges of temperature commonly met with in nature, but the present status of the problem does not warrant any attempt at a closer approximation. Indeed, it seems almost certain that the magnitude of the temperature coefficient will be found to vary, not only with different plant forms and with different stages of development of the same form, but also with the values of the temperatures considered. It is readily conceivable that the relation which we are seeking may be determined satisfactorily only by the use of a temperature coefficient which is itself a variable, changing in value with the progress of the organism through its life cycle and with the annual march of the seasons, as well as with variations in the temperature itself. The time is not yet ripe, however, for even an *a priori* discussion of this matter.

Having tentatively established the temperature coefficient which is to be taken as a measure of the effectiveness of temperature in advancing growth (the intensity factor), we must make a similar assumption in case of the duration factor. For what period of time should we apply our assumed temperature coefficient? This question is precisely the same as the one met with in connection with direct temperature summations, and its answer, as in that case, must involve the relation of time to the developmental stages in the organisms concerned. Since it is here desired to deal with the whole matter in the broadest and most general way, it is requisite to fix upon a time period, which will, as nearly as possible, approximate the period of active growth in the majority of higher plants. As one of the authors<sup>23</sup> has emphasized, the controlling climatic conditions are primarily effective for most plants only during the season of active growth. This growing season may be approximated, for phytogeographical purposes, as the average or normal length of the frostless season, the number of days which intervene between the average date of the last killing frost in spring and the first in autumn. This has been adopted as the time factor in the present study.

<sup>23</sup> Proc. Amer. Phil. Soc. 1913; as already cited.

Since the individual data from which was derived DAY's chart<sup>24</sup> of the average length of the frostless season in the United States have not been published as such, the average lengths of the frostless season for the various stations in the United States with which we have been concerned have been taken from the 106 climatic summaries by sections,<sup>25</sup> published by the Weather Bureau.

Not only is it necessary to fix upon the length of time during which temperature integration is to be made, it is also requisite, for each station, to establish the month and day with which the summation is to begin, and likewise that with which it is to terminate. These dates must of course be chosen with reference to the beginning and ending of the period of active growth. The first is here taken as the date *next following* the average date of the last frost in spring, and the second as the average date of the first frost in autumn. If the dates for the beginning and end of the assumed growth period were determined from daily mean temperatures (as in case this period were taken as beginning with the first day in the year having a daily mean of 40° F. or above, and ending with the last day having this temperature), then they should bear a definite relation to the real first and last dates of the active period. Such methods have been frequently used in phenology. By our method, the beginning and end of the average period of active growth are determined from frost data rather than from mean daily temperatures. The two methods would probably prove equally satisfactory, with no great discrepancy in most cases.

The geographical distribution of plant associations and of species involves the effectiveness of climatic features throughout many years, and a knowledge of these features for any single year is of but little value in the present connection. This consideration involves still another sort of climatic integration that has not yet been mentioned here; namely, the averaging of the effective conditions for long periods of time. This operation has been implied, however, in the preceding treatment of the average length

<sup>24</sup> DAY, P. C., Frost data of the United States, etc. U.S. Dept. Agric., Weather Bureau, Bull. V. 1911.

<sup>25</sup> Summary of the climatological data of the United States, by sections. U.S. Dept. Agric., Weather Bureau. No date. These pamphlets appear to have been prepared about 1909-1910. The data generally extend through 1908 or 1909.

of the frostless season. For the intensity factor of temperature effectiveness it is necessary to employ as data, not the temperature of any single instant, nor the mean temperature of any single day, but what has been termed the *normal mean* temperature for each day of the frostless season. Fortunately, these data have been calculated by BIGELOW and have been made available through publication by the U.S. Weather Bureau.<sup>26</sup> These have been here employed.

The plan of this study is: (1) to sum the normal daily mean temperatures of each station considered, for the period of the average frostless season; (2) to sum the temperature efficiencies corresponding, respectively, to the normal daily means and to the adopted coefficient for 10° of variation; (3) to plot both sets of temperature indices so obtained in the form of charts; and (4) to compare the form and location of the climatic areas or zones thus exhibited.

### The temperature indices

As in the case of direct temperature summations, so in that of the summation of temperature efficiencies, it is necessary to establish a temperature which may be taken as a starting-point. This should approximate the temperature at which general plant growth is evident and should be chosen according to the same criteria as are employed by phenology in similar cases. For both series of summations we have taken the rate of growth and development as unity with a daily mean temperature of 40° F. (4.4° C.). Normal daily means below 40° F. do not occur in BIGELOW's tables. If they did occur, growth on such dates would be taken as nil, on the basis of our assumptions.

For each of the direct summations, the normal daily mean *minus* 39, for the date next following the average date of the last frost in spring, is taken as the first term. To this are added the normal daily means, each decreased by 39, for all dates up to and including the average date of the last frost in autumn. Practically, the summation of the unmodified normal daily means was first made

<sup>26</sup> BIGELOW, F. H., The daily normal temperature and daily normal precipitation of the United States. U.S. Dept. Agric., Weather Bureau, Bull. R. 1908.

for the period and then a quantity equal to 39 times the number of days in the average frostless season was subtracted from the sum.

For the summations of temperature efficiencies, the normal daily efficiencies corresponding, respectively, to the normal daily means of BIGELOW's tables have simply been added for the same days as in the direct summations, thus giving what may be termed a tentative index of temperature efficiency for growth during the normal frostless season. We shall term this the *efficiency index* and the direct summation will be called the *direct index*. It is these two indices and the charts formed from them that are to be compared.

To obtain the daily temperature efficiencies corresponding to the various normal daily temperature means as the latter are given by BIGELOW, it is necessary merely to deduce them from our basic assumption, namely, that the growth rate is unity at 40° F., and that it doubles for each rise of 10° C. (18° F.) above this. We shall employ the Fahrenheit scale, not because it is in any way as satisfactory as the Centigrade, but because temperature observations in the United States and the published data deduced therefrom have the antiquated form.

If  $t$  be taken as the normal daily mean temperature on the Fahrenheit scale, and if  $u$  be the corresponding temperature efficiency for growth, according to our assumption, then

$$u = 2^{\frac{t-40}{18}}.$$

It is clear that this equation fulfils the assumed conditions, that the efficiency doubles with each rise of 18° F., for, if  $t$  is 40° F., then  $u$  is unity; if  $t$  is 58° F., then  $u$  is 2; if  $t$  is 76° F., then  $u$  is 4; and so on. It becomes necessary, therefore, merely to interpolate between the already known values the various values of  $u$  corresponding to the actual values of  $t$  which are to be dealt with. The equation just given may as well be written

$$\log u = \frac{\log 2}{18} (t - 40),$$

from which the requisite values of  $u$  may readily be obtained. Another way of stating the above relation is this: The tempera-

ture efficiency ( $u$ ) for any Fahrenheit temperature ( $t$ ) is the eighteenth root of 2 effected with an exponent equal to the given temperature *minus* 40. From this the values of  $u$  may be directly calculated. Only one cycle of eighteen quantities, however, needs to be so determined in any case, for the coefficients of the second cycle are double the corresponding ones of the first, etc.

The following table presents the approximate values, derived as above, for the efficiencies corresponding, respectively, to the temperatures (in whole degrees) from 40° to 99° F.

APPROXIMATE EFFICIENCY INDICES FOR TEMPERATURES, IN WHOLE DEGREES, FROM 40° TO 99° F., ASSUMING THE EFFICIENCY TO BE UNITY AT 40° AND TO DOUBLE WITH EACH RISE IN TEMPERATURE OF 18 DEGREES

Temperature, degrees F. ( $t$ )	Efficiency ( $u$ )	Temperature, degrees F. ( $t$ )	Efficiency ( $u$ )	Temperature, degrees F. ( $t$ )	Efficiency ( $u$ )
40.....	1.0000	60.....	2.1603	80.....	4.6662
41.....	1.0393	61.....	2.2451	81.....	4.8490
42.....	1.0802	62.....	2.3331	82.....	5.0396
43.....	1.1226	63.....	2.4245	83.....	5.2384
44.....	1.1666	64.....	2.5198	84.....	5.4424
45.....	1.2123	65.....	2.6192	85.....	5.6568
46.....	1.2599	66.....	2.7212	86.....	5.8782
47.....	1.3096	67.....	2.8284	87.....	6.1090
48.....	1.3606	68.....	2.9391	88.....	6.3496
49.....	1.4142	69.....	3.0545	89.....	6.5972
50.....	1.4696	70.....	3.1748	90.....	6.8566
51.....	1.5273	71.....	3.2986	91.....	7.1258
52.....	1.5874	72.....	3.4283	92.....	7.4048
53.....	1.6493	73.....	3.5629	93.....	7.6960
54.....	1.7142	74.....	3.7024	94.....	8.0000
55.....	1.7815	75.....	3.8480	95.....	8.3144
56.....	1.8512	76.....	4.0000	96.....	8.6412
57.....	1.9240	77.....	4.1572	97.....	8.9804
58.....	2.0000	78.....	4.3206	98.....	9.3324
59.....	2.0786	79.....	4.4902	99.....	9.6980

### Efficiency indices and direct indices for the mean frostless season in the United States

All of the climatological calculations involved in this study have been carried out, and at least once repeated, on a computing machine.<sup>27</sup> The stations employed (179 in number) are those of

<sup>27</sup> The authors wish here to acknowledge with thanks that they have had the assistance, at various points in this work, of Mrs. EDITH B. SHREVE, Mr. H. E. PULLING, and Mr. J. W. SHIVE. It is also a pleasure to state that the study was made possible by the Department of Botanical Research of the Carnegie Institution.

BIGELOW's paper already cited, with a few omissions on account of the lack of corresponding frost data. Each series of summation indices was placed upon a large map of the United States, and the map was then divided into areas by isoclimatic lines, in the usual way. Thus were obtained two charts, one showing the geographic range of the efficiency indices and the other the corresponding range of the direct ones.

It was at once observed that the relations of the different areas to one another are surprisingly similar in the two charts, so that one chart appears about as valuable for bringing out the temperature characteristics of phytogeographic areas as does the other. A detailed study of the two charts, however, made it clear that they differ, as regards the relative form and relative numerical characteristics of their respective zones, in many particulars. The two charts are here presented as figs. 1 and 2. The numerical efficiency data from which these charts were derived (with the aid, at certain points, of probabilities based upon topographic contour lines represented on the original maps<sup>28</sup>) are each placed near the position of the corresponding station, the latter represented by a small circle (for the names of the stations employed, see BIGELOW, *loc. cit.*). To compare the two series of indices, the ratio of each direct index to the corresponding efficiency index was obtained, thus giving a ratio for each station. These ratios have been placed upon a map in a manner quite similar to that used in charting the indices themselves, and the resulting chart, with its isoclimatic lines, is presented as fig. 3.

The interpretation of our charts as regards plant distribution will not be taken up here. They are to be studied, of course, in connection with DAY's chart of the average length of the frostless season, with the charts given by B. E. LIVINGSTON, and with any vegetation map of the United States which it is desired to interpret along these lines.

As has been mentioned, the charts of figs. 1 and 2 show a marked similarity in the form and position of the isoclimatic lines which are represented. Of course it is at once to be observed that the direct summation indices are uniformly much larger than the

<sup>28</sup> U.S. Geol. Survey contour map of the United States, 18X28 in.

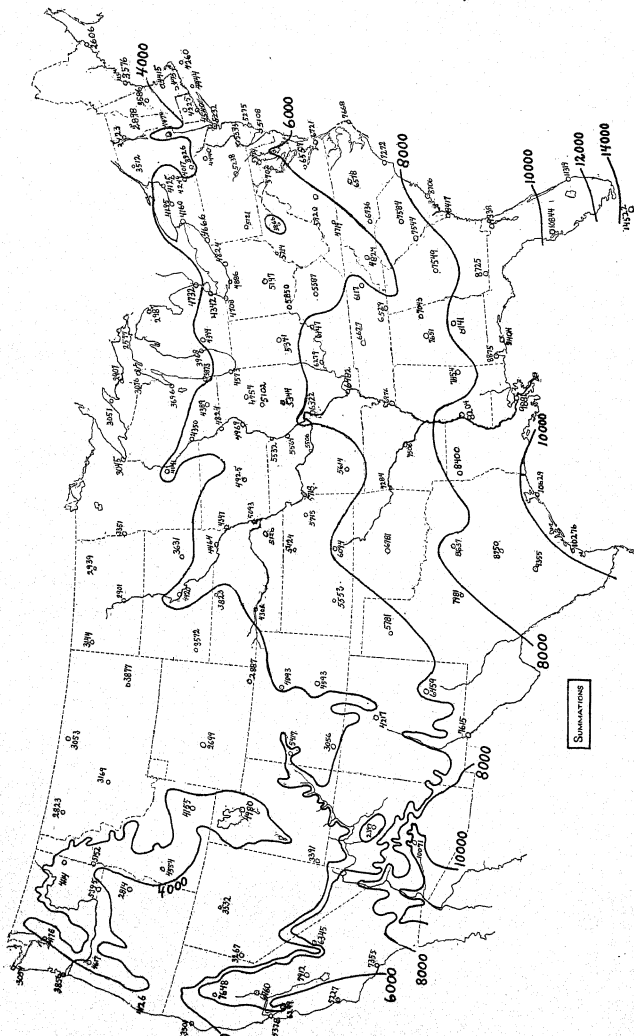


FIG. 1



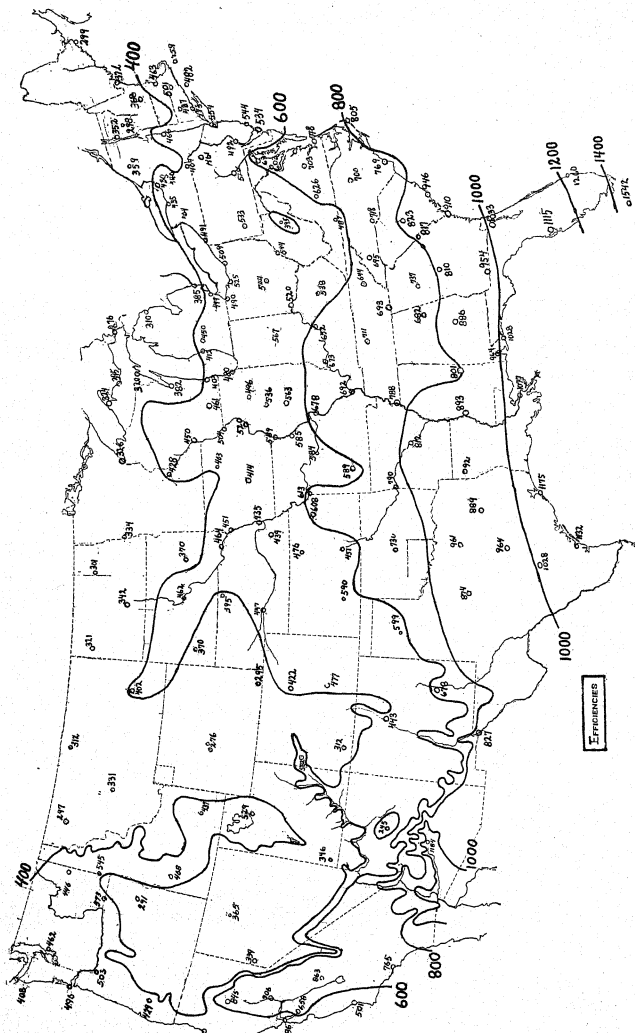


FIG. 2

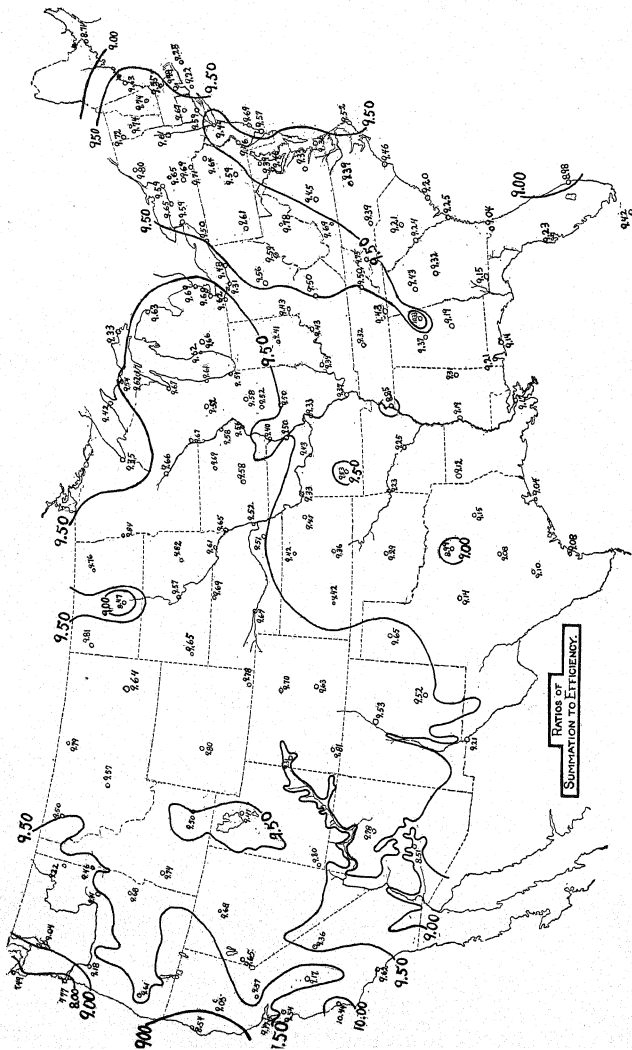


FIG. 3

corresponding ones of the other series, and simple inspection of the two charts seems to indicate that the former indices are rather uniformly about ten times as great as the latter. No ratio between these two indices really exists which is common to all our stations, but what we wish first to emphasize is that the two charts are outstandingly and even surprisingly alike in the general form of their isoclimatic lines. The stations for which data are available are so irrationally distributed over the country (being relatively closely crowded in the eastern part, with low climatic gradients, and widely separated in the west, with high gradients) that an average ratio, or any other statistical function of the ratio series, must be without meaning, but it is clear from the ratio chart (fig. 3) that ratios between 9.00 and 10.00 characterize almost the entire area of the United States. In other words, for the great majority of stations the magnitude of the ratio in question does not depart more than 5 per cent from the value 9.50. Considering that all our original data must be regarded as only roughly approximate, this appears to indicate a very good agreement between the results of the two methods. We may state, therefore, that *for most of the area of the United States*, the two methods here employed for estimating temperature effectiveness for plant growth give results which agree within the limits of a plus or minus variation no greater than 5 per cent. It will be noted that this statement practically involves the placing of the phenological method of direct summations upon a much more satisfactory basis than this method has heretofore possessed; it now appears that the direct summation method gives the same *general* form of chart as does the other method of temperature integration, and this empirical treatment really ascribes to the former method some degree of the theoretical foundation upon which the latter rests.

The similarity between our two summation charts, however, is only approximate and superficial. If it held rigidly, either of the two charts would suffice for both, and any efficiency index might be deduced from the corresponding direct index, merely by dividing the latter by the proper constant. The efficiency indices are not uniformly ten times the summation indices, nor is there any ratio other than ten which may be assumed with the aim of attaining

greater uniformity of detail in the two charts. The numerical data indicate, furthermore, that it would be quite impossible to make one chart more like the other by choosing other values through which to pass the isoclimatic lines.

The most striking difference between these charts (figs. 1 and 2) is seen in the basin and Pacific regions. Nowhere on the Pacific coast does the efficiency index fall below 400, while the northern half of this coast characteristically exhibits direct indices below 4000, Tatoosh Island, Washington, having a direct index as low as 3054. Thus it has been impossible to draw the lines on the chart of direct summations so as to bring the northern area with indices above 4000 (from Great Salt Lake to the Columbia River) into conjunction with the southern area having the same sort of indices (California, Arizona, etc.). On the efficiency chart these two areas become joined, however. Other less striking quantitative differences are clearly enough indicated, and this sort of inspection establishes the fact that the ratio of the direct summation index to that of efficiency can by no means be regarded as constant for the whole country. It is of course to be remembered that the hypsometric map has been called into requisition in the placing of our isoclimatic lines, the stations for which data are available being far too few and too far apart to give the detailed information which such charts require. Nevertheless, it seems perfectly clear that while the isoclimatic lines may really have other positions than the ones here assigned them (it is safe to suppose that almost every centimeter of these lines would be displaced to some extent if more data were at hand), yet the discrepancies between the two charts are not primarily to be related either to lack of data or to careless or inefficient interpretation of the information at hand.

Our arithmetical treatment of this question of the value of the ratio of the direct index to the index of efficiency brings out the fact that this ratio varies, for the stations employed, from 7.49 (Tatoosh Island, Washington) to 10.44 (San Luis Obispo, California) or 10.33 (Anniston, Alabama). As has been mentioned, the values of these ratios are presented in fig. 3, where lines are shown for the ratio values 8.00, 9.00, 9.50, and 10.00. This chart brings out the geographic distribution of these various values.

What may be the meaning of this new kind of climatic chart, as regards vegetational distribution, plant activity, or any other phenomena influenced by climatic conditions, cannot yet be surmised, but the chart does indicate a very important truth as far as climatology and climatological methods are concerned. If we look upon the normal daily mean temperature as in some way involving a criterion for the approximate evaluation of the naturally effective heat supply, it is shown that the summation of these normals for the period of the frostless season does not give, excepting in a very superficial way, the same chart for the United States as does the corresponding summation of temperature efficiencies following the assumptions here made.

It may be said that the direct index is a measure of one dimension of the temperature factor of a climate, while the efficiency index as here employed measures another dimension. Which of the two dimensions more nearly approximates the measure of the temperature effectiveness of a climate, as far as plant growth is concerned, will no doubt remain for a long time undetermined. The present independent status of the direct summation method for the treatment of temperature data rests upon phenological observations in the open, while the status of the other method of treatment (employing temperature efficiencies instead of the daily mean temperatures themselves) is founded upon deductions from the chemical velocity coefficient of VAN'T HOFF and ARRHENIUS, upon physiological experimentation under more or less controlled conditions, and upon the fact that all physiological processes are chemical in their nature or else depend upon other processes which are chemical. It must be admitted, on physiological grounds, that some sort of efficiency summation seems likely to prove more truthful and more valuable in vegetational-climatic studies than the direct summation of temperatures. The latter method of treatment has no a priori or logical basis (although, as has been seen, it is not altogether without pragmatic or empirical points in its favor), and appears, superficially at least, to be quite arbitrary in its theoretical conception.

The details of the distribution of our various ratio values deserve attention, for they bring out quite unequivocally, not only that

these values do indeed vary, but also that their variations are in accordance with the geographical positions of the stations concerned. It is clear from fig. 3 that the portions of the United States characterized by ratios below 9.00 lie mainly near the margins of the country. The lowest ratios of our series occur in western Washington. Other areas with ratio values below 9.00 are indicated for northwestern California, southeastern California and southwestern Arizona, southeastern Florida, northeastern Maine, and north-central North Dakota. It thus appears that a line representing a ratio of 9.00 may be passed, according to the indications of fig. 3, around the area of the United States, entering within its boundaries only sufficiently to include the areas just mentioned.

On the original chart, from which fig. 3 is taken, were drawn lines representing the ratio values by tenths, from 9.0 to 10.0. From this chart (as of course also from the numerical data given in fig. 3) it is evident that the ratio values with which we are dealing are consistently distributed, with few or no exceptions. An area of high ratios occurs in the Appalachian Mountains, having the highest value at Anniston, Alabama (10.33). Another similar area occupies the Rocky Mountains and appears to extend northward to the Canadian boundary, with maximum values of 9.81 (Williston, North Dakota and Durango, Colorado). A small area of high values lies about Moorehead, Minnesota (9.84), and Huron, South Dakota (9.82). A less pronounced but strongly and consistently indicated area occupies the southern peninsula of Michigan and eastern Wisconsin, the maximum here being at Port Huron, Michigan (9.69). Finally, a somewhat questionably indicated area of high ratio values centers about San Luis Obispo, California (10.44). Interior areas of low ratios require no discussion; they are few, and are generally based upon the evidence of single stations.

From the study of this chart it seems clear that the ratios of direct temperature summations, for the normal frostless season, to the corresponding efficiency summations as here derived, must be considered as a measure of some climatic characteristic. Just what this characteristic may depend upon we are not here attempt-

ing to find out; it obviously has to do with the order of magnitude of the normal daily mean temperatures and also with the distribution of these magnitudes throughout the mean frostless season. There seems little doubt that a mathematical treatment involving limits may bring out the nature of those characteristics of the frostless season which are measured by the ratio as employed in this paper.

### Conclusions

1. The method of direct temperature summations has proved itself to give, *in a broadly general way and for most of the area of the United States*, nearly the same climatic zones as does our method of efficiency summations; for practical purposes and for the present, the former method, till now based solely on phenological observations, seems thus to be placed in closer logical connection with the temperature coefficients of chemical, physical, and physiological processes than has heretofore been the case.

2. The similarity between the results derived by these two methods of temperature integration, however, is only superficial and roughly approximate. The ratios of direct summation to efficiency summation range in magnitude, for the mean frostless season in the United States, from a minimum of 7.49 to a maximum of 10.44.

3. A rational and consistent climatic chart represents the geographical distribution of these ratio values; on such a chart the marginal regions of the country are frequently characterized by low ratios and the two main mountain systems appear to control areas of high values.

4. There seems to be no doubt that the ratio here brought forward quantitatively represents a climatic dimension or characteristic, which appears to be some sort of function of the daily normal temperatures upon which this whole study has been based and of the time distribution of these temperature data within the period of the mean frostless season.

## APOGAMY IN ATAMOSCO

LULA PACE

(WITH PLATES XIII AND XIV)

*Atamosco texana* Greene (*Zephyranthes texana*) is one of the Amaryllidaceae, and is called amaryllis, atamosco lily, or rain lily. The so-called rain lilies in this region bloom in the summer and autumn for a few days after each rain. This one is not quite so well known here as the white lily, *Cooperia Drummondii*, which is about twice the size and much more abundant in this locality; but 100-200 miles farther west *Atamosco* is the more plentiful.

The first collection of material was made for class use, as the ovules and embryo sac are very large; but very early in the preparation of this material interesting things were noticed. So the material on hand was carried to Bonn and worked over there so far as the stages permitted. I regret exceedingly not being able to complete it at that time under the direction and with the suggestions and criticisms of Professor STRASBURGER. But it gives me great pleasure to acknowledge my indebtedness to him for his kindly interest and stimulating suggestions during the first part of the work.

### Material

The material was all collected in one locality, near Waco, Texas; and in this particular place *Atamosco* is quite abundant over two or three acres. But as the flowers are solitary and often precede the leaves, it is not easy to find young stages. I now have plants under cultivation and hope to get these desired stages later.

Two collections have been made, one in 1909 and the other in 1911. The intervening year I was abroad and no collection could be made. The material was fixed in chromacetic acid (0.25 per cent chromic acid and 1 per cent acetic acid). In some cases about 10 drops of 1 per cent osmic acid was added to 50 cc. of the above. Alcohol-acetic (three parts absolute alcohol and one part acetic acid) and alcohol-formalin (two parts of 50 per cent alcohol and one

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part of 10 per cent formalin) were also used. The triple stain, safranin, gentian violet, and orange G, and iron hematoxylin were found most satisfactory.

### Pollen

The pollen is quite normal in every way in the anthers sectioned (fig. 1). The tube nucleus is usually somewhat amoeboid in shape and occasionally has more than one nucleolus. The generative cell with its nucleus and bit of cytoplasm about it is quite small. In this figure the generative nucleus has the chromosomes segmented. The number is 12 and was counted in several other grains in the same anther. But in the majority of the pollen grains studied the generative nucleus was in the spirem stage (fig. 2). In this same anther a few grains were seen with possibly two generative nuclei; but it is probable they are the sperm nuclei, as they are both in the same cell. A germinating pollen grain from the stigma shows the generative nucleus in mitosis and in advance of the tube nucleus (fig. 3). In fig. 3, *a* this nucleus is shown with greater magnification, and the remainder of this nucleus, which was in the adjacent section, is shown in *b*. The chromosomes show longitudinal splitting and there are 12.

### Embryo sac

The mother cell is only slightly larger than the adjacent cells when first distinguishable (fig. 4). The ovule now increases rapidly in size and the mother cell becomes very large. The division of the mother cell has not been seen. Many ovaries with ovules showing large mother cells have been cut, and many that were apparently the same size had mature sacs. The development of the sac must be very rapid, for several ovaries had mature sacs and mother cells apparently occurring together without any definite order. But other ovaries showed all ovules in the same stage of development; one in particular has every ovule with the nuclei of its sac in the third and last division, with all stages of mitosis from well developed spirem to late telophase.

The two-nucleate (fig. 5) and four-nucleate sacs show nothing unusual. The spirem for the next division has the normal appearance. In figs. 6 and 6, *a* the anaphase stage of the two nuclei from

the micropylar end of a four-nucleate sac are shown. The entire figures being found in two adjacent sections, both are drawn separately, as the number of chromosomes is so large that it is difficult to reconstruct the two figures and make one accurate drawing. In one nucleus the spindle is almost parallel with the section. In this spindle at one end 11 chromosomes appear, and the same end in the next section shows 13, giving 24 chromosomes at one end of this spindle. It is not possible to make an accurate count at the other end, but there seems to be about the same number. The other nucleus is almost perpendicular to the section and at one end the 24 chromosomes are unusually distinct. A metaphase from the chalazal end of another four-nucleate sac is shown in fig. 7. It also shows the 24 chromosomes. A similar one might be given from the micropylar end of another sac. A somatic cell taken from the walls of the ovule is given for comparison (fig. 8); it shows 24 chromosomes. Fig. 9 is a telophase of this same division from the micropylar end of the sac. The old spindle of the previous division is still quite distinct, but less so than the spindle of this division. This spindle shows wall beginnings in the one parallel with the sections. Only one end of the other spindle appears in this section, as it is perpendicular to it. But this nucleus, which already has the nuclear membrane developed about it, shows approximately 24 chromosomes.

The 8 nuclei arrange themselves in the usual fashion, egg apparatus, antipodals, and two polars that fuse, each group being very distinct and almost diagrammatically organized. The filiform apparatus is well developed, but not so strikingly as in *Parnassia* (20a). The indentations so noticeable in the *Parnassia* synergids are not often seen at all in *Atamosco* (fig. 10). It was present in a few instances, but was never a deep notch. The only noticeable feature of the sac is the large nuclei containing little other stainable material than the large nucleolus. There are often two nucleoli in the egg and occasionally in the synergid nuclei also. They both stain red in the triple stain, or if stained in the iron hematoxylin, they are apparently alike. The antipodal end of the sac has the usual organization (fig. 11). The three antipodal cells are definitely organized, but are without cell walls. The polars are in contact in

the center of the sac. Fig. 12 shows the egg apparatus after the pollen tube enters. One male nucleus is within the egg near the nucleus, the other is just below it. The primary endosperm nucleus lies just above the antipodals and the fusion of the polars seems to be complete. Fig. 13 shows about the same stage. In this case one male nucleus is still not in contact with the egg, while the other has already fused with the polars, showing only as a bit of very dense nuclear material in the upper part of the primary endosperm nucleus, in which all the nuclei are still evident (fig. 13, *a*). Somewhat earlier stages were found with the polars still quite distinct and the male nucleus some distance away. The adjacent ovule shows a similar condition to that in fig. 13, *a*. Another sac in which the pollen tube has entered is shown in fig. 14. The sperm nucleus is not in contact with the egg nucleus, but the other sperm nucleus has begun fusing with the almost completely fused polars (fig. 14, *a*). Here both sperm nuclei have very dense masses of chromatin, looking like a very thick spirem except for thin places at intervals. These thin places seem to be only very delicate threads which with slight magnification are not easily seen, and the nuclei then have the appearance of having the chromosomes already segmented. In fig. 15 the micropylar end of a sac is given with the male nucleus just in contact with the egg nucleus, but there is no evidence that it is fusing, not even a flattening of either nucleus. The triple fusion is already completed and the spirem is forming for the first division. One part of this nucleus is very much denser than the remainder of it and is probably where the male nucleus entered. The very large nucleolus seems to be budding. In another sac the male nucleus lies directly in front of the egg nucleus, but is not in contact with it, while the primary endosperm nucleus of this sac has completed the first division. Here the wall has formed, but it quickly disappears, for the endosperm contains many nuclei before permanent walls appear. The male nucleus does not always come in contact with the egg nucleus, or if it does, it moves away, as it is not in contact in older stages (figs. 17-23).

About 600 sacs have been seen in which the sperm nucleus is within the egg. In very few instances are they in contact, and these were always the earlier stages. The flowers probably always

wither within 24 hours after opening. Those brought in and pollinated had all done so. These withered flowers show the male nucleus within the egg.

Fig. 16 shows an early prophase in the division of the egg. The spirem develops in the usual way, and is very long. Fig. 17 shows the segmentation completed, with a very pale nucleolus and approximately 24 chromosomes. The male nucleus is at some distance from the egg nucleus and shows no change. Fig. 19 shows a similar stage. In Fig. 20 the nuclear membrane has disappeared. About this stage the male nucleus begins to disintegrate (fig. 18); but sometimes the spindle is forming with the membrane of the male nucleus apparently still intact (fig. 21). A spindle is shown in fig. 22, and here the male nucleus is disorganizing. A late anaphase of the egg has the male nucleus entirely without a membrane (fig. 23). The chromatic material is organized into approximately 12 masses, which seem to be more or less perfectly formed chromosomes, although there are evident signs of disintegration. There is nothing in the egg or in this nucleus itself to suggest the possibility of its being a cell of the embryo. This suggests the possibility of "merogonie."

A somewhat earlier anaphase shows approximately 24 chromosomes at each end of the spindle, and the somewhat elongated male nucleus apparently in contact with the spindle (fig. 24). This sac has four endosperm nuclei, two near the base of the sac and two near the middle. Fig. 25 is that of an undivided egg. The pollen tube has the three nuclei in it. There are about 64 endosperm nuclei in the sac and but little trace of the synergids in the adjacent sections. Other sacs in this ovary have embryos with about 16 cells. It is probably a case of a delayed pollen tube and the primary endosperm nucleus dividing before fusion with the male nucleus, although this is the only instance seen where that seems probable. But as has been said above, most of the material of this stage was pollinated in order to be sure of fertilization. It is also possible for this to be the second pollen tube to enter this sac. But if so, all trace of the other one has disappeared.

The endosperm nuclei often contain 7 or 8 nucleoli, all of which stain a brilliant red in the safranin-gentian-violet combination.

An endosperm tissue 2-4 cells in width, but which does not completely fill the sac, and with many free nuclei still present, is found with the embryo of 30-40 cells. There is such a mass of long curved chromosomes in the endosperm that no satisfactory count could be made from any of my preparations. In several instances one could count 50 and know there were still others. One such nucleus, which was in three adjacent sections, is shown in figs. 26, 26, *a*, and 26, *b*. It seems clearer to show each section separately than to reconstruct the nucleus from the three sections. Here more than 60 chromosomes can be counted in all three sections, but it is certain that there were several cut chromosomes, as some were evidently shorter than others, and in this plant the chromosomes all seem to be approximately the same length. The number would be 60 from the fusion of two polars with 24 each and a sperm nucleus with 12. It is possible that in some cases the polars only enter into the fusion, which would give 48. But the triple fusion seems the common condition in the material examined. This is probably the fourth division of the endosperm of this sac. These divisions are not always exactly simultaneous; for in one instance in the second division one nucleus has completed the mitosis, the new nuclei being already perfect, while the other nucleus was still in the anaphase.

### Embryo

The embryo shows nothing unusual in its development. The first division has already been described, and is shown in figs. 20-24. One two-celled embryo was seen with a vertical wall, but in all others it was the usual horizontal wall (fig. 27). This figure shows the pollen tube entering one synergid.

### Abnormal sacs

*Atamosco* also has many sacs in which the arrangement of the nuclei does not follow the usual form. These sacs have the antipodal group in the micropylar end of the sac and egg apparatus at the side. The organization of the two groups of cells is as perfect and as characteristic as if they were in the usual position. About 2000 ovaries have been cut; many were too young to show mature sacs, and of others no record was kept. But in about 300 ovaries

that were mature, there were side eggs in 56 and in these 56 ovaries there were 205 of these abnormal cases. The other sacs in these ovaries had nothing unusual in their nuclear arrangement. In the majority of these abnormal sacs there was nothing unusual except the position of the cells, and only those with cells in unusual positions were included in the above statements.

Fig. 28, however, shows two eggs in the micropylar end of the sac with no synergids. The whole of this sac was not seen, so just what the arrangement of the other cells was could not be determined. Both of these eggs may develop, for a sac was seen with two young embryos, one of about 6 and the other of 10 cells, located exactly as these two egg cells were, and with endosperm of 30-40 nuclei. Two other sacs with embryos similar to this were seen. Occasionally more than two eggs may be organized in a sac (fig. 29). This sac has 5 cells in the micropylar end, all of which look like eggs, and two of which show intermediate characters and may function as synergids. This leaves only one antipodal and two polars which have already fused and lie very near the antipodal. Two eggs and two synergids are sometimes found in the one egg apparatus. Sometimes more than one egg apparatus is organized. One was seen with two at the side of the sac, in addition to the usual micropylar apparatus. Not all of this sac was seen, but it has at least 12 nuclei in it. The egg apparatus in these side positions is usually normal in all other particulars; but in one case in which it is at the side near the antipodal end of the sac, the egg is turned vertically, while the synergids have themselves oriented so as to bring their apices toward the edge of the sac.

What might be called a double sac is shown in fig. 30. There are 15 nuclei present, and it is possible there should be 7 instead of 6 in the micropylar group, making 16 altogether, but in tracing from section to section I could not be quite sure. Fig. 31 is from a sac that is almost normal. It seems to have 4 antipodals, and an egg apparatus of one synergid and one egg some little distance from the micropylar end of the sac. The sperm nucleus is in contact and just behind the egg nucleus. A sperm has probably fused with the polars, as there is a dense portion in this otherwise almost clear nucleus. In many of these sacs the antipodal end contains no

nuclei unless the egg apparatus approaches that region. Fig. 32 is from a sac with 13 nuclei, 7 of which have the organization of the antipodals at the micropylar end of the sac, 4 are in the lower part of the sac near the antipodal region, two synergids and two eggs, while the two polars are the only normal feature of the sac. The pollen tube is entering the egg apparatus.

As was said above, in the large majority of these abnormal sacs there are the usual 8 nuclei, and these have the usual organization, but the groups are not in the usual position. Fig. 33 shows a common arrangement except the antipodals here are not exactly at the micropylar end. The egg apparatus is perfect (fig. 33, *a*), as are the polars and antipodals, each group showing its characteristic differentiation. A four-celled embryo in a similar sac is shown in fig. 34. Here the primary endosperm has not divided. PORSCH (21) and those holding the archegonium theory would regard these extra groups as the organization of additional archegonia. If the two groups of cells usually found in an embryo sac are archegonia, the interchange of characteristic organization would not be very remarkable. In the 16-nucleate sac of *Euphorbia procera*, MODILEWSKI (15) figures 4 groups of 4 nuclei each. From each of these groups one nucleus moves to the center to form the primary endosperm nucleus, leaving 3 in each of the groups. The micropylar group he calls the egg apparatus, and that one is the only one figured as functioning. But each of the groups at the side of the sac have the appearance of an egg apparatus.

### Discussion

The egg apparatus is almost diagrammatic, so the first thing to attract attention was the very small sperm nucleus as compared with the egg nucleus. But as the egg nucleus contains very little stainable material except the nucleolus, while the sperm nucleus is very dense, the real condition was not suspected, for the sperm nucleus is often much smaller than the egg nucleus. LAND (14) figures a very small but dense spiral sperm in *Silphium*. NAWASCHIN (17) shows a similar one in *Helianthus*. GUIGNARD gives a like condition in *Iris* (7), and in some of the Ranunculaceae (9) and Solanaceae

(10); and STRASBURGER (27) figures a small more or less coiled sperm for *Urtica dioica*. Less difference in the relative size of the two nuclei appears in *Cypripedium* (19). Other instances might be cited, but these are sufficient to show that a difference in the size of the sex nuclei is not unusual.

Older stages were then cut and a spirem was found in the egg nucleus while the sperm was often not even in contact with it. This was supposed to be a case of the early prophase taking place before the fusion of the sex nuclei. GUIGNARD (8) shows such a case in *Lilium Martagon*, and *Cypripedium* (19) and *Calopogon* (20) both show this condition. BLACKMAN (1), CHAMBERLAIN (3), and FERGUSON (6) have all figured early stages in mitosis before the fusion of the sex nuclei in *Pinus*. In all of these, however, the sperm nucleus was as active as that of the egg, both nuclei being in approximately the same stage. But in *Atamosco* only the egg nucleus formed a spirem (fig. 16). It soon began to seem strange that there was no increase in the size of the sperm and no evidence of fusion. So more and more material of this stage was cut. In all cases where the pollen tube had entered the sac this condition was seen. More than 600 examples like figs. 12-15 were found, and about half as many in which the spirem in all stages is formed in the egg nucleus (fig. 16). Two-celled embryos show no trace of this sperm nucleus (fig. 27); and a few spindles and telophase stages of this division also give no trace of it.

Then young ovaries were cut to get the development of the sac. But not many had been collected and no satisfactory chromosome count could be made. In one ovary the third mitosis was in progress, but unfortunately the material was not well fixed, some plasmolysis having taken place. Yet in two nuclei of the metaphase stage more than 12 chromosomes were certainly present; but in both instances the nuclei were in the chalazal end of the sac; so this might only mean a repetition of the condition STRASBURGER (25) found in *Lilium Martagon*, of which he says:

Die Schwesterchromosomen bei der homöotypischen Teilung des im Chalazae der Embryosakanlage (die hier direkt aus der Embryosakmutterzelle hervorgeht) befindlichen Kerns, statt wie sonst, ohne Längsspaltung in die Kernspindel eingefügt zu werden, eine solche Längsspaltung, wie bei



einem typischen Teilungsschritt erfahren, was eine Verdoppelung der Chromosomenzahl zur Folge hat.

This was the state of the problem when I left Bonn.

A new collection was made this last autumn. Even from this collection the division of the mother cell has not been obtained. As was stated above, the development of the sac must go on very rapidly, for in some ovaries certain ovules are in the mother cell stage while others have mature sacs; and these seem not to be related to any part of the ovary or to follow in any special succession; they occur apparently without any order. The buds appear at the surface of the soil one day with the mother cell undivided and the flowers are open the next and usually last only one day; so the whole development must go on very rapidly.

There is no evidence that permanent walls came in at the division of the mother cell. The two-nucleate sac (fig. 5) never shows any evidence of disintegrating megaspores; so it belongs to the *Lilium* type described by COULTER and CHAMBERLAIN (5, p. 73), in which these two nuclei are the daughter nuclei without separating walls, and the nuclei of the four-nucleate sac are the four megaspore nuclei without separating walls. A discussion of this question was given in the paper on *Cypripedium* (19), and also by COULTER (4). A recent paper by BROWN and SHARP (2) shows this condition, and the same view is presented.

The third division in the sac I was fortunate enough to get in nearly all the ovules of one ovary. In many of these the chromosomes were quite distinct and several counts were made (figs. 6, 7, 9). In all cases approximately 24 chromosomes could be counted. The sporophyte number is 24 (fig. 8); therefore the reduction division seems not to have taken place. This gives the egg the sporophyte or diploid number of chromosomes; and, according to STRASBURGER's definition, when such an egg develops an embryo without fertilization it is a case of apogamy. He says (26, p. 80):

Daher ich das Kriterium für echte Parthenogenesis in der Weiterentwicklung eines Geschlechtsproduktes erblickte, das mit der ihm normal Zukommenden haploiden Chromosomenzahl anhebt. Wenn ein diploides Ei einen keim bildet, so bezeichnete ich dessen Entwicklung als Apogam, das Ei selbst als apogames Ei.

He also says that such a diploid egg is in reality already fertilized, und zwar in dem Sinne, dass der Zustand, den die Befruchtung in seiner Mutterpflanze schuf, in ihm noch fort dauert, da er nicht durch den Vorgang der Reduktionsteilung, gewissermassen durch Entfruchtung, aufgehoben wurde.

It is already from its organization endowed with the double number of chromosomes, so it goes on, so far as the chromosome number is concerned, developing like the cells of a bud or an adventive embryo. The development of these *Anlagen* would hardly be compared to genuine parthenogenesis, in spite of the fact that no fertilization takes place here. But WINKLER used a different terminology, based on a somewhat different conception, especially with reference to the importance of the haploid or diploid number of chromosomes. He (29) would call this "somatic parthenogenesis." He defines parthenogenesis (p. 11) as

die apomiktische Entstehung eines Sporophyten aus einem Ei, und zwar (a) somatische parthenogenesis, wenn das Ei einen Kern mit der diploiden, unreduzierten Chromosomenzahl besitzt (b) generative parthenogenesis, wenn der Kern des Eies mit der haploiden Chromosomenzahl ausgestattet ist.

He defines apogamy as

die apomiktische Entstehung eines Sporophyten aus vegetative Zellen des Gametophyten, und zwar (a) somatische Apogamie, wenn die Zelle oder der Zellkomplex, die den Sporophyten liefern, in ihren Kerne die diploide Chromosomenzahl besitzen (b) generative Apogamie, wenn die Kern der Mutterzellen des Sporophyten nur die Haploide Chromosomenzahl führen.

That is, WINKLER would call the development, without the aid of a male gamete, of an egg (either haploid or diploid) parthenogenesis, the development of vegetative cells (from a gametophyte) apogamy. There can be no question concerning the latter, but there may be a difference of opinion as to whether the development of both diploid and haploid eggs without fertilization should be called parthenogenesis.

In *Atamosco* the organization of the egg, with the exception of the presence of the diploid number of chromosomes, is perfect so far as morphological evidence goes. The whole egg apparatus is characteristically developed, being quite distinct in appearance

from all other cells of the sac. Even the synergids are not to be confused with the egg, each showing its own peculiar characters (figs. 10, 11). It happens that more than one egg is sometimes organized, and apparently all these eggs in *Atamosco*, but no other cell, may function. As all the nuclei of the female gametophyte have the diploid number of chromosomes, it might be thought that any cell could develop a sporophyte, and therefore adventive embryos and polyembryony would be common. But no evidence of adventive embryos was found; and only a few sacs with two embryos were seen, and in every instance they were paralleled in other sacs by an egg in a similar position (figs. 28, 33, 34).

If the egg be considered a diploid gamete, then this should be considered parthenogenesis, but we usually define a gamete as a cell that fuses with another before developing, and it would seem that it should at least be capable of so doing if it is called a gamete. But this egg cell lacks this gamete character; for *Atamosco* seems to show conclusively, not only that it is capable of developing without fertilization, but that it will not fuse with the male gamete although the two nuclei be in contact (figs. 15-24). If this inability to fuse shows that it is really not a gamete, then this is apogamy. It is certainly apogamy if we consider it from the standpoint of the part taken in the process by the male nucleus. Apogamy might be defined as the development of a sporophyte from a diploid egg without fertilization. Vegetative apogamy would be the development of a sporophyte from vegetative cells of the gametophyte, while parthenogenesis would be reserved for the development of a sporophyte from an egg with the haploid number of chromosomes.

In 1900 JUEL (11) showed that *Antennaria alpina* has very few staminate individuals, and even these have anthers entirely sterile or only slightly fertile. The megaspore develops without the reduction division. The *Alchemilla* group was investigated by MURBECK (16) in 1901 and by STRASBURGER (23) in 1905. In the apogamous species the reduction division does not take place in the megaspore development. In the microspore series, in some species, the cells develop no farther than the mother cell stage, others disintegrate after the first division, while still others complete the tetrad division and imperfect pollen is produced. In

1904 OVERTON (18) investigated *Thalictrum*, finding the pollen normal. In some instances there was a reduction division in the development of megaspores, while others developed without this, a case exactly parallel to that of *Marsilia* as shown by STRASBURGER (24) in 1907. In *Taraxacum officinale* JUEL (12, 13) in 1904 and 1905 states that the embryo sac mother cell divides only once, the inner daughter cell developing the sac, but no reduction division takes place. The reduction division does take place in the microspore development.

ROSENBERG (22) in 1907 found in *Hieracium* that the reduction division takes place in the microspore development, but in some species, at least, they are disintegrating even at this time, and the mature anthers are empty. In some species the megaspore development is occasionally normal, giving a haploid gametophyte; but it usually happens that some sporophyte cell (epidermal, chalazal, or nucellar cell) produces the embryo sac. So this is a case of apospory in the development of the sac; and as a diploid egg is organized, it is also a case of apogamy in the development of the embryo. The work of WINKLER (28) in 1904 and of STRASBURGER (26) in 1909 with *Wikstroemia* shows reduction in the microspore development with but little pollen maturing, while reduction does not take place in the megaspore development. In 1910 STRASBURGER (27) added *Elatostema* to the apogamous angiosperms. It also has the reduction in the pollen development with much irregularity in the pollen, some anthers producing pollen of normal appearance. The mother cell of the ovule begins the reduction division but returns to the vegetative mitosis; but in some cases reduction does take place. From these it seems that reduction always takes place in the microspore development, the failure being only in the megaspore series, and in some species it is only partial here.

It will be seen that normal pollen is reported only for *Thalictrum*, some species of *Taraxacum*, and *Atamosco*, the others all having little pollen or defective pollen. The female gametophytes for four genera are diploid, while in the other three both haploid and diploid female gametophytes are developed; but it is not known that these haploid eggs develop parthenogenetically. According to

YAMANOUCI (30) this is true for the fern *Nephrodium*, which develops a haploid sporophyte from a haploid unfertilized egg.

## SUMMARY OF APOGAMOUS ANGIOSPERMS

	Pollen	Female gametophyte
Urticaceae		
Elatostema .....	Defective	Diploid or haploid
Ranunculaceae		
Thalictrum .....	Normal	Diploid or haploid
Rosaceae		
Alchemilla } .....	Defective	Diploid
Eualchimilla }		
Thymelaeaceae		
Wikstroemia .....	Little	Diploid
Compositae		
Antennaria .....	Little	Diploid
Taraxacum .....	Normal or little	Diploid
Hieracium .....	Little	Haploid or diploid
Amaryllidaceae (Leucojaceae)		
Atamosco .....	Normal	Diploid

STRASBURGER (24) holds that a diploid egg not only is not fertilized, but is not capable of being fertilized. He says (p. 166):

Womit kann nämlich das apogame Ei einer *Marsilia* besser beweisen, dass es weder Befruchtungsbedürftig noch Befruchtungsfähig ist, als dass es den Spermatazoiden den Eintritt in das Archegonium unmöglich macht? Die diploide Chromosomenzahl bedingt es also, dass im Ei das Befruchtungsbedürfnis sich nicht einstellt und damit auch der Reiz wegfällt, der die Tätigkeiten sonst anlost, ist welche die Befruchtung vorbereiten. Also kommt doch wohl eine grundsätzliche Bedeutung an dieser Tatsache zu, dass nicht die einfache, sondern die doppelte Chromosomenzahl im Kern vertreten ist.

In the apogamous *Marsilia* the canal cell does not disintegrate, so that the sperm cannot enter the archegonium. STRASBURGER's idea of the incapability of the fertilization of the diploid egg was worked out on theoretical grounds altogether, as is shown in the quotations just given, and as he said in discussing this question with me.

OVERTON (18, p. 278) says that in pollinated material he often found pollen tubes in the micropyles of ovules and even in contact with the egg, and also the fusing of the second sperm nucleus with the endosperm nucleus. But his preparations failed to show the fusion of the sperm and egg nuclei, and he adds:

An diesem Grunde darf ich auch nicht positiv behaupten, dass zur normalen Keimentwicklung stets Befruchtung notwendig ist, wenn auch alle sonstigen Tatsachen und die Beobachtungen an Pflanzen im Freien keinen Zweifel darüber lassen, dass Befruchtung stattfinden kann, wenn das Ei die reduzierte Zahl der Chromosomen führt.

In *Atamosco* I have been able to settle this question beyond a doubt I think; for about 30 cases of an egg dividing with the sperm inactive although in contact, or nearly so, with the egg nucleus were examined. The sperm gradually disintegrates (figs. 18, 21-24, 27) and evidently the egg is incapable of fusing with the sperm, the latter being perfectly normal in every way so far as appearances go, for the sperm had the haploid number of chromosomes, and the pollen seemed entirely normal, and pollen tubes enter the sac in the usual way. The other sperm does fuse with the polars either before or after their fusion. This would seem to show that the triple fusion is not of the same character as that which takes place when the sperm and egg fuse. So *Atamosco* seems to prove STRASBURGER'S theory that the diploid egg is incapable of fertilization.

On the completion of the work, I reported to Professor STRASBURGER what I had found, and in his reply, received February 25, 1912, he says:

Die Schwerpunk Ihrer Arbeit wird darin liegen, dass der Eikern, weil er doppeltchromosomig ist, die Aufnahme dem Spermakern versagt, ungeachtet die übrigen Einrichtungen bei der Pflanze fortbestehen, die den Spermakern bis in das apogame Ei hineinführen.

### Summary

1. The pollen is normal. The haploid number of chromosomes is 12.
2. The third division in the embryo sac shows the diploid number of chromosomes to be 24.
3. Usually the ordinary 8-nucleate sac is organized, but occasionally antipodals organize in the micropylar end and the egg apparatus at the side of the sac, sometimes more than one egg organizing.
4. Two male nuclei come into the sac with the pollen tube, one

fusing with the two polars, the other entering the egg but never fusing with it, and finally disintegrating during the first division in the egg.

5. A diploid egg seems to be incapable of fertilization.

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#### EXPLANATION OF PLATES XIII AND XIV

All figures were drawn with a camera lucida. The magnification given is that of the original drawings, which are reduced one-half in reproduction. The abbreviations used are as follows: *e*, egg; *g*, generative nucleus; *m*, male nucleus; *p*, pollen tube; *s*, synergid; *t*, tube nucleus.

FIG. 1.—Pollen grain: tube nucleus (amoeboid) and generative nucleus with 12 chromosomes;  $\times 1000$ .

FIG. 2.—A spirem in the generative nucleus, from the same anther;  $\times 1000$ .

FIG. 3.—A germinating pollen grain from the stigma; the generative nucleus in advance of the tube nucleus and in mitosis;  $\times 400$ .



FIG. 3, *a*.—The generative nucleus from fig. 3;  $\times 1000$ .

FIG. 3, *b*.—The remainder of the above nucleus from the adjacent section; chromosomes show longitudinal splitting; 12 chromosomes in *a* and *b*;  $\times 1000$ .

FIG. 4.—Diagram of young ovule showing mother cell;  $\times 600$ .

FIG. 5.—Two-nucleate sac; daughter nuclei without separating walls;  $\times 400$ .

FIGS. 6 and 6, *a*.—Two adjacent sections of the same sac; both nuclei of micropylar end of sac in anaphase stage; in each spindle 24 chromosomes can be counted at one end; an accurate count at the other end is not possible;  $\times 1000$ .

FIG. 7.—Metaphase at chalazal end of four-nucleate sac with 24 chromosomes;  $\times 1000$ .

FIG. 8.—A somatic cell from ovule walls in metaphase, showing 24 chromosomes;  $\times 1000$ .

FIG. 9.—Telophase of the third division of the sac; remains of previous spindle; the uncut spindle of the present division shows the beginnings of wall formation; the cut spindle is perpendicular to the section, so only one end of it is shown; this nucleus shows approximately 24 chromosomes;  $\times 1000$ .

FIG. 10.—The egg apparatus from mature sac; filiform apparatus well developed, but no notch in the synergids;  $\times 1000$ .

FIG. 11.—Antipodal end of sac; polars in contact;  $\times 400$ .

FIG. 12.—Micropylar end of sac after pollen tube enters;  $\times 600$ .

FIG. 13.—Micropylar end of the sac;  $\times 1000$ .

FIG. 13, *a*.—Primary endosperm of same sac before triple fusion is complete;  $\times 1000$ .

FIG. 14.—Egg and male nucleus almost in contact;  $\times 1000$ .

FIG. 14, *a*.—Triple fusion from same sac;  $\times 1000$ .

FIG. 15.—Micropylar end of sac;  $\times 600$ .

FIG. 16.—Egg nucleus with well developed spireme and male nucleus near it;  $\times 1000$ .

FIG. 17.—Egg nucleus showing approximately 24 chromosomes; male nucleus near it;  $\times 1000$ .

FIG. 18.—Micropylar end of sac; egg nucleus with approximately 24 chromosomes; the nuclear membrane has disappeared;  $\times 1000$ .

FIG. 19.—Egg nucleus similar to that in fig. 17;  $\times 1000$ .

FIG. 20.—Egg nucleus similar to that in fig. 18, but sperm still has nuclear membrane;  $\times 1000$ .

FIG. 21.—Spindle beginning to organize in the egg; male nucleus very dense;  $\times 1000$ .

FIG. 22.—Metaphase in the egg nucleus; nuclear membrane of the male nucleus breaking down; 1000.

FIG. 23.—Anaphase in egg nucleus; male nucleus has approximately 12 chromatic masses, apparently chromosomes;  $\times 1000$ .

FIG. 24.—Micropylar end of sac; male nucleus very close to spindle of egg nucleus, which is in anaphase stage;  $\times 600$ .

FIG. 25.—Undivided egg with about 64-nucleate endosperm; other sacs in this ovary have embryos of about 16 cells; pollen tube contains three nuclei;  $\times 600$ .

FIGS. 26, 26, *a*, and 26, *b*.—Three sections of the same endosperm nucleus in metaphase;  $\times 1000$ .

FIG. 27.—Two-celled embryo with pollen tube entering one synergid;  $\times 600$ .

FIG. 28.—Micropylar end of sac with two eggs and no synergids;  $\times 600$ .

FIG. 29.—Diagram of sac with three, possibly five, eggs, one antipodal, and two fusing polars;  $\times 105$ .

FIG. 30.—One antipodal in the usual position, the group of six nuclei in the micropylar end of the sac having the appearance of antipodals; a perfect egg apparatus is organized on one side; one cell near the antipodal region has the egg organization, and four nuclei are fusing in the upper end of the sac;  $\times 105$ .

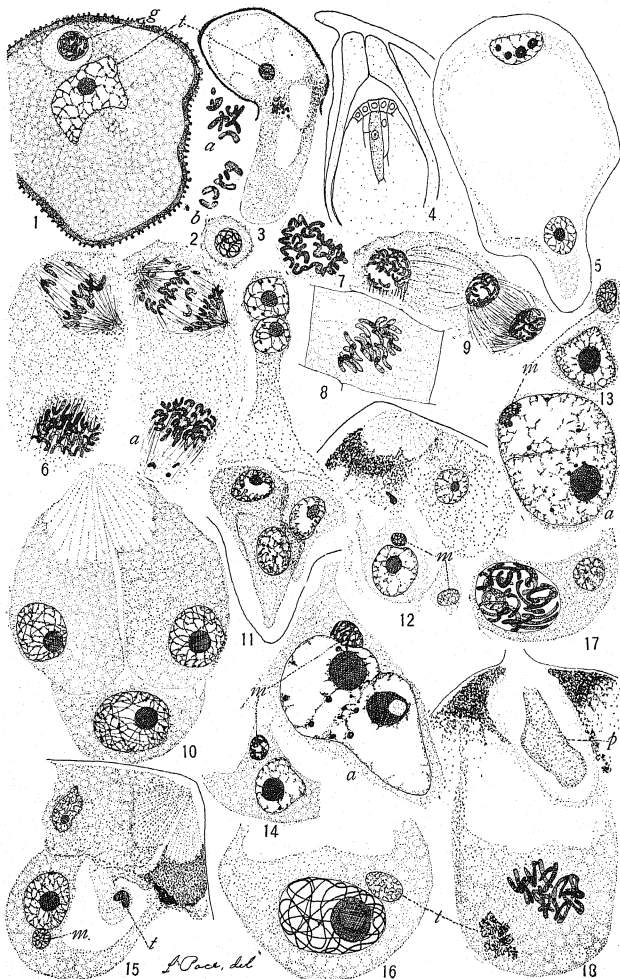
FIG. 31.—Egg apparatus slightly to one side, showing male nucleus in the egg, behind and just in contact with the nucleus;  $\times 600$ .

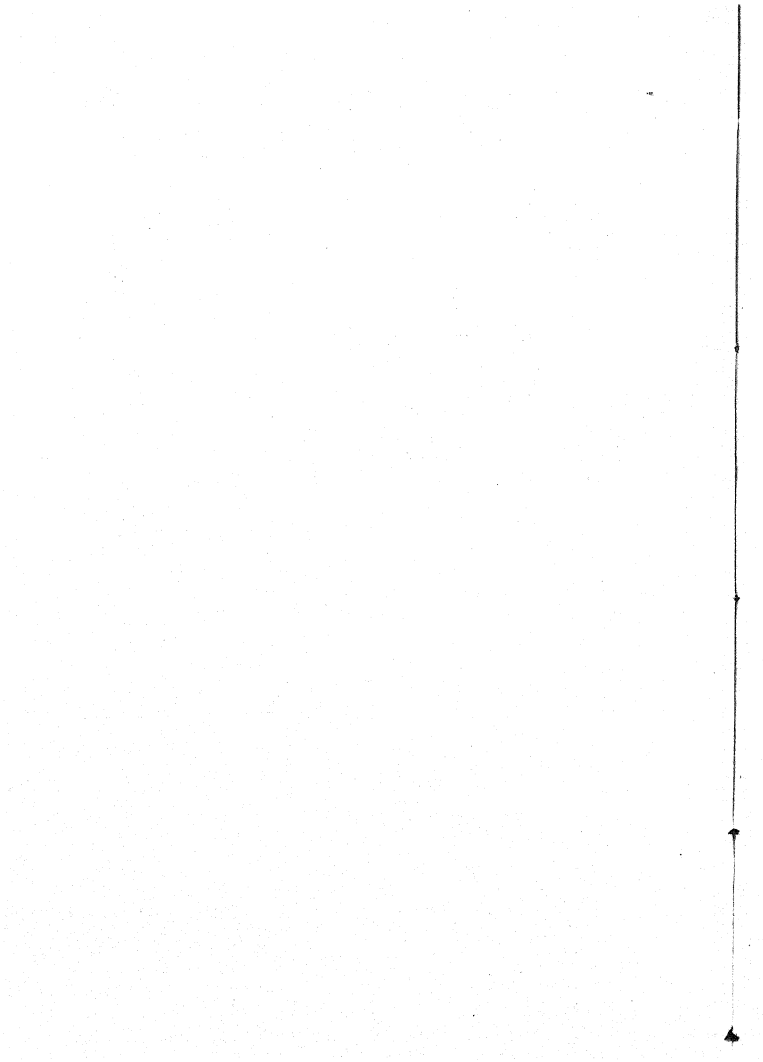
FIG. 32.—Egg apparatus of four nuclei near the antipodal region; pollen tube entering;  $\times 1000$ .

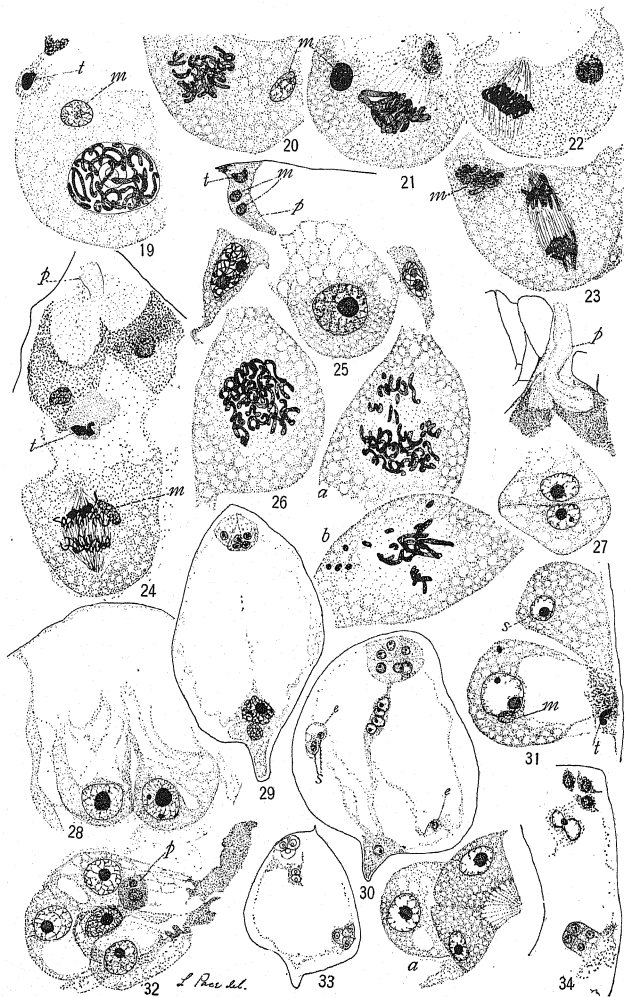
FIG. 33.—The very common type of abnormal sac except that the antipodals are not exactly at the micropylar end of this sac;  $\times 105$ .

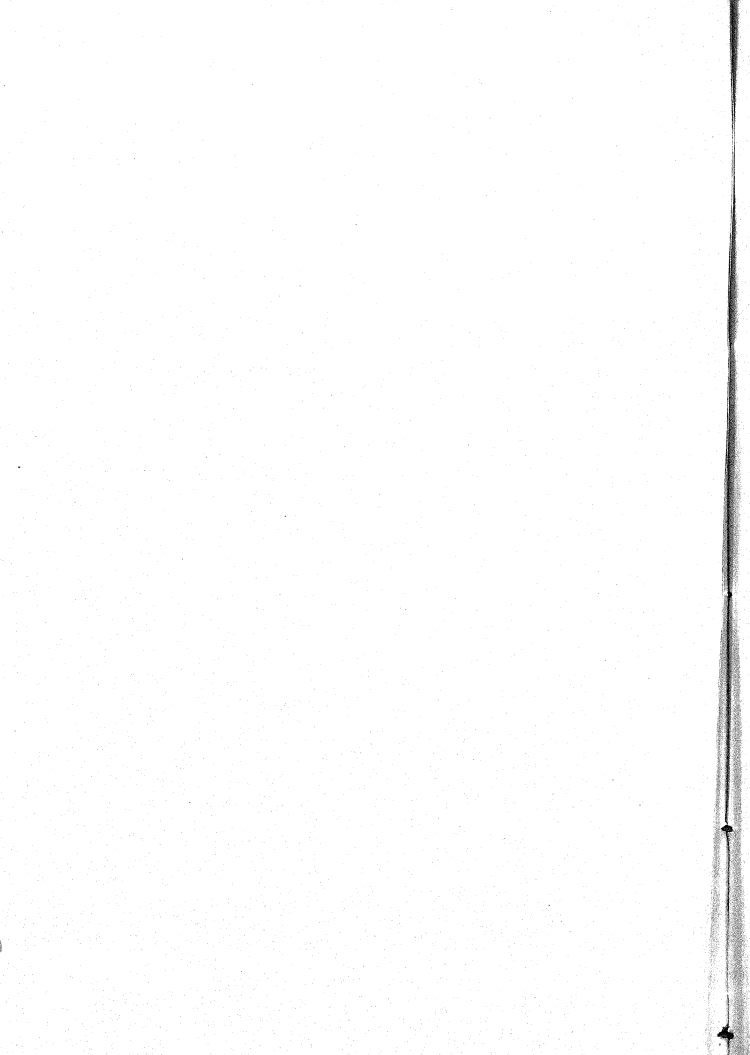
FIG. 33, *a*.—Egg apparatus of the same sac;  $\times 600$ .

FIG. 34.—Four-celled embryo from a side egg; primary endosperm nucleus still undivided;  $\times 105$ .









## THE TEPARY, A NEW CULTIVATED LEGUME FROM THE SOUTHWEST

GEORGE F. FREEMAN

(WITH ELEVEN FIGURES)

The great aridity and extreme heat of the southwestern part of the United States have developed a flora singular in type and striking in contrast with such as is found in the more humid sections. So widely distinct are the atmospheric conditions that none but the hardiest and most adaptable of introduced plants are able to withstand the violent readjustment made necessary by the new environment, even though water in abundance be artificially supplied. Professor J. J. THORNBURGH of this station is authority for the statement that only a small percentage of plants or varieties developed in sections having a decidedly different climate from that of Arizona may be successfully cultivated here.

Alfalfa, which has been grown within the territory for many years, is now thoroughly acclimatized and is today our greatest single farm product. Among other crops introduced since the European settlement of Arizona and southern California and thoroughly acclimatized may be mentioned barley, the white Sonora wheat, the olive, pomegranate, and several varieties of figs, dates, and citrus fruits. Realizing that the plants which are most successful in resisting the vicissitudes and appropriating the peculiar advantages of the climate of Arizona are those which have been longest grown within its borders, the writer has had his attention drawn to some of the native economic plants of this region. Among these may be found varieties of agricultural plants which have been grown within the confines of Arizona for hundreds or perhaps even thousands of years, years when the ruins that now crumble in the desert sands were populated with a happy and prosperous race, years when the canal systems which still can be distinctly traced ran on higher levels and watered more lands than those which at the present time distribute the waters of the Gila and Salt rivers. Here among the Pima and Papago Indians, descendants

or successors of these former builders, may be found varieties of corn, beans, pumpkins, and squashes which have survived the race under whose husbandry they were produced. Centuries of adaptation have therefore produced types eminently suited to withstand the extremes of heat and drought to which the climate and indifferent agriculture of a primitive people often exposed them.

The investigations of which this paper forms a report are concerned with the varieties of native beans only. The need for a leguminous food plant to restore nitrogen to soils irrigated with pumped water, and the necessity of finding drought-resistant crops suitable for use where "dry-farming" is being attempted, has directed the attention of the Arizona Experiment Station to these beans for several years. Tests at Yuma under irrigation, and at McNeal under dry farm conditions, have already demonstrated the ability of these native beans to fulfil all of the requirements of drought-resistance and fertilizing value, and in addition they have the ability to produce profitable crops of a staple product.

During these agricultural tests, made for the most part from seeds secured originally from the Indians, it was noticed that many apparently different sorts occurred. In order to study the different varieties in their native condition, therefore, and to secure samples for testing their relative values, the writer in company with Director FORBES spent two weeks during late July and early August 1910 among the Papago Indians in their villages situated in the valley between the Baboquivari and Quijotoa mountains, some 50-100 miles southwest of Tucson. Here, in a region with 9 inches of rainfall annually, these beans were being grown successfully with no irrigation save that of a little flood water which came down the mountain washes.

During the course of this trip 32 samples of beans were secured. In addition to these, 10 samples had been previously secured by Mr. CAVILLO from the Papago Indians near Santa Rosa and grown one year at Yuma; 7 samples were furnished by Professor J. J. THORNBUR from beans secured from the Pimas at Sacaton; some 25 carefully selected samples were contributed by Mr. MENAGER, of the Indian Oasis, which he picked from beans secured from the Papagos, and finally, as a result of a three days' trip among the



Pimas at Sacaton, 16 additional samples were secured. This mass of material when carefully sorted yielded 71 apparently distinct sorts. These were planted on the testing grounds at the university during the summer of 1910 and produced strains of the most widely diverse types and economic values.

The first lot of seed coming into the hands of the writer were 10 samples obtained originally from Mr. CAVILLO at Santa Rosa, but which had been grown for one year at the Experiment Station at Yuma. In working over this material two distinct types were observed. The first type was characterized by a larger size, averaging about 0.23 gm. When present at all, the markings or flecks were much coarser; there was an absence of lines radiating from the hilum, such as are used by botanists to distinguish between the kidney and lima beans. Another distinguishing characteristic of this group was the smoother, more glistening surface of the seed coat. The second type averaged distinctly smaller (about 0.15 gm.); the markings or flecks when present were much finer; distinct lines radiating from the hilum were frequently present; the surface of the seed coat lacked the characteristic glossiness of the first type. Inquiry soon developed the fact that Indians and Mexicans of southern Arizona and Sonora distinguish sharply these two classes, the latter designating the first type as "frijoles" and the second as "teparies." It is interesting to note that they think of these two legumes as being quite as distinct as we should think of rye and wheat. After once becoming familiar with the differences between the two types, anyone recognizes without hesitation and at a glance whether any given sample is composed of teparies or frijoles, in spite of the fact that previously he may never have seen that particular variety.

### Frijoles

The common frijole undoubtedly belongs to *Phaseolus vulgaris* Linn. The Papago equivalent of the bean is *mōn*. They call a certain yellow bean *sōam mōn* (*sōam*=yellow, *mōn*=bean). In like manner they call the pink bean *yura mōn*. The word *yura* is of Yaqui origin and is said to refer here to the white man rather than to the color of the bean, thus meaning "the white man bean."

This also accords with the Indian tradition that beans had been secured from the white man, but that they had grown teparies "long time." Distributional and other evidence also points to the more southern origin of beans, which in all probability were brought northward by the earlier Spanish settlers and missionaries.

### Teparies

The name tepary or *tepari* (Spanish) originated from the Papago words *stüle päve*; *stü-te* meaning white and *päve* having reference to the kind of plant to distinguish it from the *mön* or bean. In like manner they speak of *sōam* (yellow) *päve* and *spüle mōök* (muddy) *päve*. In addition to the very common statement, above quoted, that they (the Indians) had secured beans from the white man, but had grown *päve* a long time, several of the old men and women of the Papago tribe in widely separated villages gave the strikingly uniform answer that their forefathers got teparies from a people which had once inhabited that region (the Baboquivari and Quijotoa district of southern Arizona), and had grown these beans in the same valleys where they are now grown, but that these people went away "long ago." However, two different old women said that they remembered having gathered dark colored wild teparies during their childhood in the mountains farther south (presumably in Sonora). It is a common statement by Mexicans that the yellow tepary grows wild in Sonora and makes vines like a morning glory in the damp soil of canyons. Weight is further given to these statements and traditions concerning the local origin of these varieties by the greater resistance of teparies to extremes of climate than is possessed by beans. Adaptation through a longer series of years, or perhaps, as the present evidence seems to indicate, even domestication from the wild state in the same localities where now grown, has rendered them more hardy than the exotic beans. When the irrigating water or rains fail, teparies will frequently make a good crop when beans are a total failure. In a field of red Indian beans belonging to a Pima Indian near Sacaton in the fall of 1910, in which the crop was a total failure on account of an insufficient water supply, the writer noted a few yellow tepary plants growing as an admixture among the beans. Under conditions such

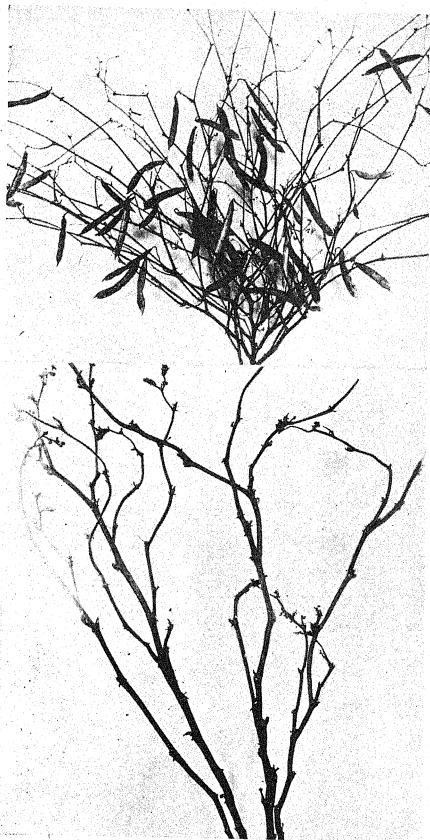


FIG. 1.—Tepary above, bean below: both planted May 17, photographed August 20; tepary producing abundantly; bean crop total failure on account of heat; leaves removed to show presence or absence of pods.

that the most vigorous of the beans had produced but two or three pods, some of the tepary plants had as many as 200 ripe pods and the average was more than 100. During 1911 pink beans which were planted May 17 had not bloomed on account of the heat as late as August 20, although they have been well irrigated during that time (fig. 1). Teparies planted alongside the beans on the same day and given identical treatment had by that time ripened an abundant crop of seed. It is a common statement among the Pima Indians that when the beans are destroyed by insects the teparies go free.

It has now been noted that the traditional origin of the bean and the tepary are distinct, that physiologically the tepary differs from the bean in being more resistant to heat, drought, and insect attack, and hence is more productive in hot regions. It remains yet to describe those morphological distinctions which compel the conclusion that the tepary does not belong to the same species as does the kidney bean.

### Characters of the tepary

The characters of tepary are best indicated by contrasting them with those of the kidney bean (*Phaseolus vulgaris*) and the lima bean (*P. lunatus*). The seeds are smaller, averaging about 0.15 gm. instead of about 0.23 gm. for beans and 0.50 gm. or more for limas. When present at all, the markings or flecks on teparies are much finer than on the other two. The seed coat of the tepary lacks the characteristic glossiness of the kidney bean.

The length of the petioles of the first pair of aerial leaves are strikingly different in the tepary and the varieties of *P. vulgaris* and *P. lunatus*. Measurements of the mature petioles of the first pair of aerial leaves on a large number of seedling of several varieties of each gave for the average of the tepary 4.3 mm., for beans 24.33 mm., and for the limas 43.7 mm. The first pair of aerial leaves of all three species are simple, but those of the tepary differ from the others in having truncate instead of cordate bases (figs. 2 and 3). They are also smaller and narrower, the averages being as shown in table I.

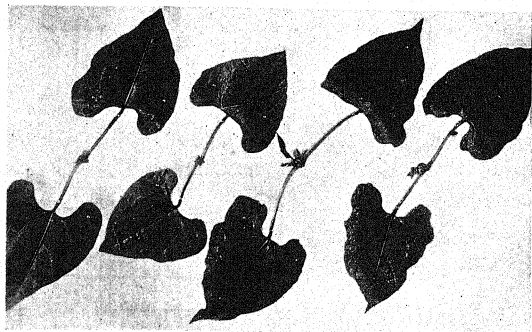
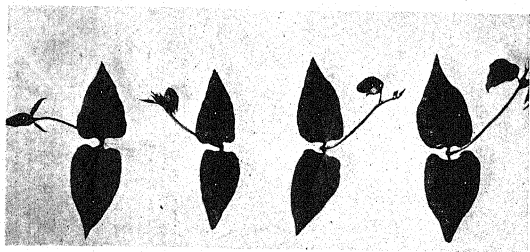
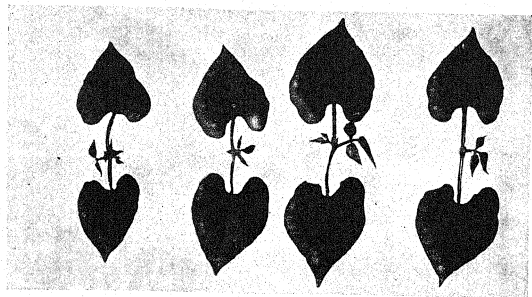


FIG. 2.—Seedlings showing first pair of aerial leaves; top, bean; middle, tepary; bottom, lima bean.

TABLE I

	Average length mm.	Average width mm.	Ratio $\frac{W.}{L.}$
Tepary.....	55.12	33.14	1.67
Kidney bean.....	47.32	45.86	1.01
Lima bean.....	72.80	66.89	1.08

The apices of all the leaves of the tepary are more acute than those of either the kidney or lima beans, and they are also much smoother (not crumpled and rough).

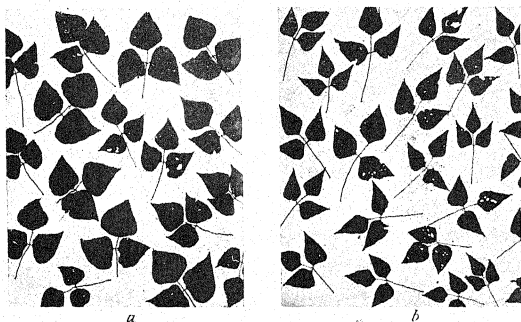


FIG. 3.—Typical leaves of bean (a) and tepary (b)

The length and breadth of the terminal leaflets of 100 normal mature leaves taken at random and including several varieties each of both beans and teparies were measured with the following results:

TABLE II

	Average length mm.	Average breadth mm.	Ratio $\frac{L.}{B.}$
Teparies.....	63.80	36.59	1.74
Beans.....	78.80	64.70	1.21

The flowers of the tepary are smaller than those of the bean, the wings are narrower in comparison with their length, and the

banner is more strongly reflexed in flower. The calyx tube is deeper in the tepary, and the two upper teeth are united to form one prominent acuminate tooth rather than being separate and nearly obscure as in the bean. The lower three teeth of the calyx tube of the tepary are also much longer and more tapering than those of the bean, being described as acuminate rather than acute triangular to obtuse or rounded as in the latter species (fig. 4). The bracts subtending the calyx of the bean are large and nearly

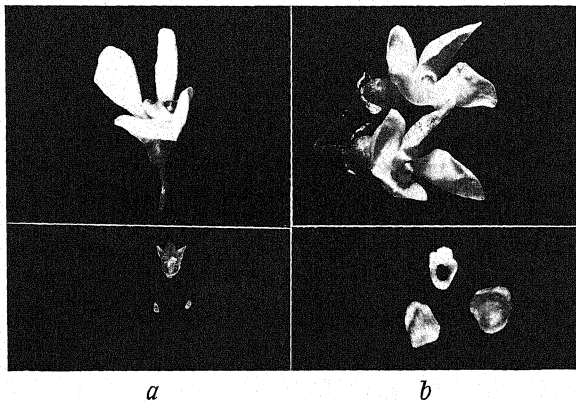


FIG. 4.—Flowers of tepary (a) and bean (b) above; dissected calyx tube and bracts of each below.

orbicular, measuring about 5 mm. in either direction. The corresponding bracts of the tepary are exceedingly small, inconspicuous, and early deciduous. They are lanceolate in outline and are about 1.5 mm. long by 0.5–0.66 mm. broad. In this respect the tepary resembles the lima bean. The flower of the lima differs from that of the bean or tepary in having its banner of a different texture and color from the wings, since it contains chlorophyll and is a pale greenish-yellow color with a purple tinge, whereas the banner of the other two species is either white or colored in the normal way.

In all three species, at the opposite side of the hilum from the micropyle, the raphe ends with a more or less prominent elevation. In beans and teparies this elevation is completely divided into two lobes by a crease beginning at the hilum and passing between them. In the limas, on the other hand, the crease enters the elevation but does not pass entirely through it. The raphe of the lima therefore ends in a V-shaped, two-pronged elevation rather than in two rounded, completely separated elevations as in the other two species (fig. 5). The character generally used in botanical keys to distinguish between the lima and kidney bean is the presence of vascular lines radiating from the hilum. These are distinctly visible in the former, but lacking in the latter species. This character is very variable in the tepary, being prominent in some varieties and entirely wanting in others, with every degree of variation between these extremes.

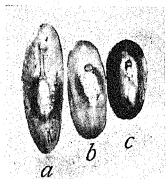


FIG. 5.—Ventral view of lima bean (a), bean (b), and tepary (c).

Since the tepary does not belong to either of the species which include the varieties of the kidney and lima beans, the question arises whether it has heretofore been recognized among the domesticated beans.

In the monographs of JARVIS<sup>1</sup> and IRISH<sup>2</sup> on the garden beans, five genera are recognized: *Phaseolus* (kidney bean), *Vicia* (broad, English, or Windsor beans), *Vigna* (cowpea), *Dolichos* (asparagus bean), and *Glycine* (soy, soja, or coffee bean). To these TRACY<sup>3</sup> adds macunna (velvet bean).

The economic species of *Phaseolus*, according to each of these writers, are three:<sup>4</sup> *P. vulgaris* (kidney bean), *P. lunatus* (lima

<sup>1</sup> JARVIS, C. D., American varieties of beans. Cornell Bull. 260. pp. 149-245. 1908.

<sup>2</sup> IRISH, H. C., Garden beans cultivated as esculents. Missouri Bot. Gard. Ann. Rept. 12. pp. 81-165. 1909.

<sup>3</sup> TRACY, W. W., JR., American varieties of garden beans. U.S. Dept. Agric., Bur. Pl. Ind. Bull. 109. pp. 5-173. 1907.

<sup>4</sup> The seven species of common bean (*P. vulgaris* Savi, *P. compressus* Martens, *P. gonospermus* Savi, *P. carinatus* Martens, *P. oblongus* Savi, *P. ellipticus* Martens, and *P. sphaericus* Martens), which were separated by GEORGE VON MARTENS (*Die Gartenbohnen*. 1860) according to the shape and size of the seed, are now recognized as varieties of *P. vulgaris* L.



bean), and *P. multiflorus* (runner bean). BAILEY<sup>5</sup> states that the moth bean (*P. aconitifolius* Jacq.) is cultivated as a human food in India, and that *P. Mungo* Linn., with its vars. *glaber* Roxberg (adzuki bean) and *radiatus* Hook., are widely cultivated in southern Asia. It has already been shown that the tepary differs specifically from *P. vulgaris* and *P. lunatus*. It may now be distinguished from *P. multiflorus*, *P. aconitifolius*, and *P. Mungo* by the following characters:

TABLE III

	Tepary	<i>P. multiflorus</i>	<i>P. aconitifolius</i>	<i>P. Mungo</i>
Leaflets..	Simple	Simple	2-3 lobed at apex	Simple
Flowers..	4-5, small, at the end of raceme which is generally inferior to the leaves	Large and showy, in many-flowered racemes	Very small, yellowish, in heads at the ends of axillary peduncles	Rather small, yellowish, in a capitate cluster of 5 or 6 on the end of stout hairy axillary peduncles
Stem ....	Nearly smooth or slightly puberulent	Minutely pubescent	Brown hairy	Densely clothed with long brown hairs

Since it is evident that the tepary does not belong to any of the species now recognized as cultivated esculents or ornamentals, there remains to compare it with the described wild species. The *Index Kewensis* lists 141 species of *Phaseolus*, of which 90 are indigenous to North and South America and the adjacent islands. ROSE<sup>6</sup> states that "nearly 50 species of *Phaseolus* have been reported from Mexico and Central America, and I have no doubt but many remain yet undescribed." Upon going over the available literature I have been able to find 54 species recorded as growing in the southwestern United States, Mexico, and Central America. Indian tradition states that the tepary has been grown within its present habitat since prehistoric times. The physiological evidence from the hardiness and drought-resistance of these plants supports this view. It is more than likely, therefore, that this species was domesticated from wild plants growing within this region or adjacent Mexico rather than from species indigenous to moist tropical

<sup>5</sup> BAILEY, L. H., *Cyclopedia of Amer. hort.* pp. 1294-1296. 1901.

<sup>6</sup> ROSE, J. N., Notes on useful plants of Mexico. *Contrib. U.S. Nat. Herb.* 5:212. 1899.

countries farther south, which were undoubtedly the original home of the less drought-resistant *P. vulgaris* and *P. lunatus*. I have been unable to secure descriptions of all of the wild species reported from Mexico and Central America, but the specific characters of the greater part of the list has been gone over very carefully.

*Phaseolus brevicalyx* Micheli (Mém. Soc. Phys. Genève 34: 261. pl. 12. 1903) is a close relative of the tepary. The latter differs from this species, however, in being glabrous or only slightly puberulent, while *P. brevicalyx* is described as pilose. It further differs strongly in the following points: petioles 2-10 cm. long (average 6.8 cm.) rather than 1-3 cm., stipules lanceolate and appressed rather than broadly ovate and spreading, calyx never punctate with black spots.

The description of *P. proriferus* M. E. Jones, though very incomplete, indicates that this also is a near relative of the tepary, but it seems to differ from the tepary in being more robust and in the rhombic-ovate, abruptly acuminate leaflets.

The following description of *P. acutifolius* Gray<sup>7</sup> seems to agree with the tepary in every particular:

*P. ACUTIFOLIUS*, sp. nov.—Volubilis; ramis gracillimis puberulis; foliolis subovato-lanceolatis e basi ad apicem usque attenuatis acutis integerrimis scabrido-puberulis; pedunculis paucifloris folio brevioribus, bracteis bracteis subulatis minimis deciduis; pedicellis flore aequilongis; calyce profunde bilabiato, labio superiore vix emarginato dentibus lateralibus et infimo ovato-oblongis subaequalibus tubo paulo brevioribus; legumine compresso lato-lineari falcato pubescente; seminibus complanatis leviter rugosis.—Mountain valley 30 miles east of El Paso; Sept.—Plant in general aspect and foliage much resembling a slender narrow-leaved variety of *P. helvolus*; but the leaves are more tapering and pointed; the 2-3-flowered peduncles are shorter than the leaf, and seldom longer than the common petiole; and the pedicellate flowers are smaller than those of *P. perennis*. It belongs to the section *DREPANOSPRON*, having a flat and falcate legume, not much curved, two inches long, a quarter of an inch wide, 8-9-seeded. Seeds oval, compressed, somewhat shining, lightly rugose. The leaflets are from one to two inches long, varying from ovate-lanceolate to lanceolate from a broad base.

Specimens of the tepary have been submitted to Dr. J. N. ROSE, who states in a letter: "I quite agree with you that your Indian

<sup>7</sup> GRAY, ASA, *Plantae Wrightianae*. pp. 43, 44.

bean is different from *P. vulgaris*, although rather closely related to it. I would suggest that you compare it with *P. acutifolius*." In a later letter he says: "We have specimens named by Dr. GRAY which look very much indeed like your specimen." Unfortunately I have been unable to obtain authentic wild examples of *P. acuti-*



FIG. 6.—*Phaseolus acutifolius* var. *tenuifolius* A. Gray

*folius*. Several plants occur in the herbarium of the university which bear this label, but they do not at all agree with GRAY's original description. Photographs of these plants and also pressed specimens of the tepary were submitted to Dr. B. L. ROBINSON of the Gray Herbarium where the type specimen of *P. acutifolius* and several of its varieties are found. The matter was turned over to

Dr. C. A. WEATHERBY, who after careful comparison with the types left by Dr. GRAY wrote as follows:

The type of *P. acutifolius* Gray is a plant collected by WRIGHT in western Texas in Sept. 1849. . . . Your plant is evidently near it, differing in its much longer petiolules of the central leaflet and in its broader leaflets. The plants [referring to the photographs] with narrow leaflets are *P. acutifolius* var. *tenuifolius* Gray [fig. 6]. There is also a second variety mentioned by Dr. GRAY in the *Plantae Wrightianae* [2:33] without name, which has leaflets larger and broader than in your plant and of which I will send a tracing.

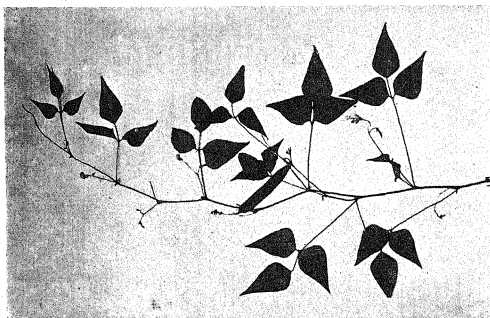


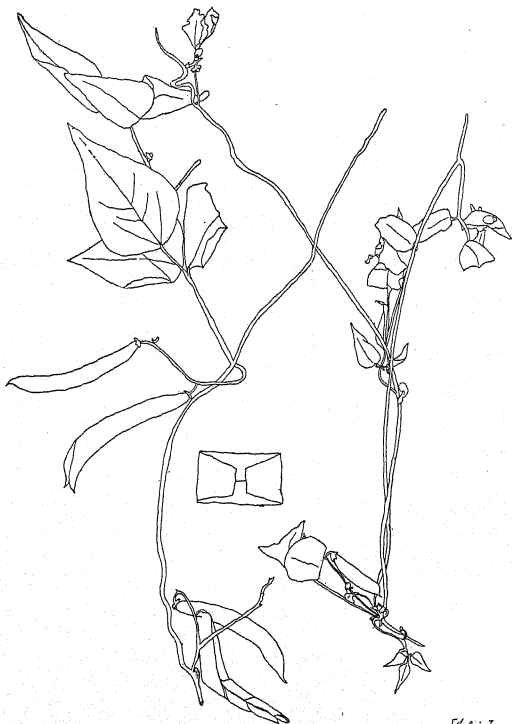
FIG. 7.—The tepary (*Phaseolus acutifolius* var. *latifolius*, n. var.)

The tracing of the type specimen of *P. acutifolius* Gray furnished by Dr. ROBINSON was found on comparison to be intermediate in form between *P. acutifolius* var. *tenuifolius* on the one extreme and the tepary on the other. The tracing of Dr. GRAY's larger, broader-leaved, unnamed variety of *P. acutifolius*, although larger than the small specimen of the tepary which I submitted to Dr. ROBINSON, was identical with the tepary as grown upon the testing grounds at this station. In fact, this tracing might have been drawn from any one of a number of herbarium specimens of the tepary which I have made during the present season (figs. 7-9).

Careful measurements of the leaves of the specimen of *P. acutifolius* found in the National Herbarium were furnished by



FIG. 8.—*Phaseolus acutifolius* A. Gray: from tracing of the type specimen made for the writer under the direction of Dr. B. L. ROBINSON, Gray Herbarium.



[folia]  
 No. 999 C. WRIGHT, Coll. M. Mex. 1861.  
*Phaseolus acutifolius* Gray  
 var. *latimbrus*

FIG. 9.—*Phaseolus acutifolius* var.: from tracing of Dr. GRAY's unnamed variety collected by WRIGHT in a valley of Sonora; tracing made for the writer under the direction of Dr. B. L. ROBINSON, Gray Herbarium.

Professor J. J. THORNER. In the following table these are compared with similar measurements of the tepary and the tracing of the broad-leaved variety furnished by Dr. ROBINSON. The measurements of the tepary were made from 100 mature leaves, taken at random from some 30 varietal plots during August 1911.

TABLE IV

	<i>Phaseolus acutifolius</i>	Tepary	GRAY's unnamed variety
Length terminal leaflet.....	40-46 mm.	40-95 (av. 64) mm.	56-71 mm.
Width terminal leaflet.....	16-21	22-52 (av. 37)	22-41
Length lateral leaflet.....	30-42	38-78 (av. 56)	42-52
Width lateral leaflet.....	12-23	20-50 (av. 33)	23-38
Length terminal petiolule.....	8-13	16-34 (av. 22)	11-18
Length petioles.....	30-40	20-100 (av. 68)	25-46

It may now be noted that the tepary leaves are decidedly larger and broader than those of the type of *P. acutifolius*, and that in the length of the petiolule of the terminal leaflet they far exceed the specific type. In most of these characters, moreover, they exceed the measurements of GRAY's unnamed variety. It must be remembered, however, that of this latter there is but one specimen, and that upon it there is only one well matured leaf whose measurements are the maximum given. In view of the above careful comparisons but one conclusion seems possible. The tepary and GRAY's unnamed broad-leaved variety are identical. Thus, in their broad leaves and robust growth they exhibit the opposite extreme of variation from the type of *P. acutifolius* that *P. acutifolius* var. *tenuifolius* does in the direction of narrow leaves and slender habit. GRAY's mention of this broad-leaved variety is as follows:

P. ACUTIFOLIUS; var. foliolis majoribus ovatis acuminatis, legumine majore (3-pollicari).—Valleys of Sonora, Sept. (949).—Plantae Wrightianae (2:33).

Although GRAY recognized it as a distinct variety, his failure to give it a name and more careful description is probably due to the fact that he had only one specimen. This lack of material I am now able to supply. I feel, therefore, that I am safe in adding to the species of *Phaseolus* which are used as esculents the name of

the tepary (*P. acutifolius* A. Gray), and suggest that this variety be called *P. acutifolius* var. *latifolius*. The description is as follows:

**PHASEOLUS ACUTIFOLIUS** A. Gray var. *latifolius*, var. nov.—Tepary.—Annual: stems recumbent, spreading or twining, 0.5–3 m. long, glabrous to puberulent: leaves smooth above, with prominent veins beneath, glabrous throughout or slightly puberulent beneath; stipules lanceolate, 2 mm. long, 1 mm. broad, striate, appressed; petioles slender, 2–10 cm. long (av. 6.8 cm.); leaflets stipellate; terminal leaflet stalked, large, 4–9.5 cm. long (av. 6.4 cm.), 2.2–5.2 cm. wide (av. 3.7 cm.), average ratio length to width 1.74, ovate to broadly lanceolate, gradually narrowed and acute at apex; petiolule of terminal leaflets 1.6–3.4 cm. long (av. 2.2 cm.); lateral leaflets same general shape as terminal leaflet but slightly smaller and inequilateral, 3.8–7.8 cm. long (av. 5.6 cm.), 2–5 cm. broad (av. 3.3 cm.): inflorescence axillary; bracts and bractlets small, deciduous: flowers medium sized, pedicellate, white or pale violet: calyx short, 3–4 mm. long, broadly campanulate, 4-toothed (upper two lobes uniting into one), teeth acuminate, pubescence on teeth and calyx scattered and fragile: banner broad, emarginate, in flower more than half reflexed, biauriculate at base, 8–10 mm. long; wings exceeding banner, obovate to spatulate, 10–15 mm. long, auriculate on one side; keel narrow, two or three turns to the spiral: stamens in two sets (9 and 1): legume 2–7-seeded (average 4.9), 5–9 cm. long (av. 7.3 cm.), 8–13 mm. wide (av. 10.5 mm.), ciliate when young, puberulent, slightly pubescent, or smooth when mature, straight or slightly curved: seeds of different varieties white, yellow, brown, or bluish black to deep violet, either self-colored or variously flecked (no red-seeded varieties have yet been observed), round oval to nearly round (as the navy bean) to strongly flattened (like a diminutive lima).—Fig. 10.

The following table gives the averages of size and shape of the seeds as determined by measurement of at least 25 normal specimens of each of 48 different strains.

TABLE V

	Length mm.	Width mm.	Thickness mm.	Ratio $\frac{L}{W}$ .	Ratio $\frac{W}{T}$ .
Maximum varietal average . . .	9.78	6.68	4.76	1.80	2.10
Minimum varietal average . . .	6.93	3.88	2.66	1.32	1.20
Average for all varieties . . . . .	8.56	5.68	3.80	1.49	1.51

COULTER<sup>8</sup> gives as the habitat of *P. acutifolius* "mountain valleys west of the Pecos, and in adjacent New Mexico and Mexico."

<sup>8</sup> COULTER, J. M., Botany of western Texas. Contrib. Nat. Herb. 2: 89. 1894.



I do not know of authentic specimens of *P. acutifolius* or its broad-leaved variety being recorded from Arizona,<sup>9</sup> but I have little doubt that a close search of the canyons of the southern part of the state will reveal its presence. The tepary is grown by the Indians and Spanish settlers throughout southern Arizona and northern Sonora. The further limit of its cultivation is not known to me, but it probably extends from southwest Texas, across New Mexico, Arizona, California, southward into northern Mexico.

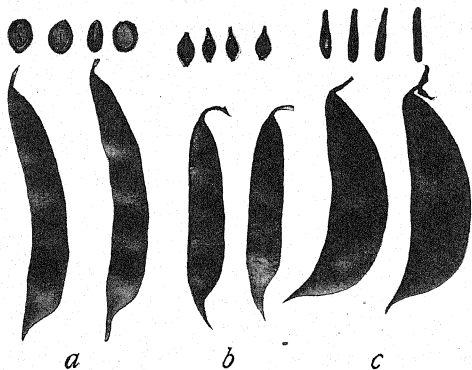


FIG. 10.—Pods of bean (a), tepary (b), and lima bean (c)

In order to discover whether the tepary has yet found its way into the hands of the commercial seedsmen, a list of the leading seedsmen of the country was secured. Especial attention was paid to making this list as complete as possible with regard to the seedsmen of the Southwest. Catalogues were then secured, and at least one sample of every named variety was ordered. Wherever from the description, or lack of description, there appeared a

<sup>9</sup> It has already been mentioned that the four Arizona specimens in the herbarium of the university, which were labeled *P. acutifolius* Gray, are *P. acutifolius* var. *lenuifolius* Gray.

possibility that the variety in question might be closely related to the tepary, it was ordered from every available source. In this manner 216 more or less distinct varieties were secured. Notwithstanding the fact that anyone familiar with the tepary could tell at a glance that none of these belonged to this group, all of those varieties which in any way resembled it were planted. In the seedling stage further confirmation of their relationship to *P. vulgaris* was made unnecessary by the uniformly long petioles of the first pair of aerial leaves.

### Varieties

Domesticated from the neighboring canyons and cultivated in small patches, attended at best by a crude husbandry, and dependent upon the precarious summer rains and uncertain floods from the mountain washes for irrigation, the tepary has lost none of its native hardiness. Like other cultivated plants, however, it has responded to domestication in the production of a number of more or less distinct varieties. These varieties manifest themselves chiefly in the color of the flowers and in the shape and color of the seed. The habit of growth, foliage, and pod characters show but little change, with the exception that the white-seeded sorts seem to have slightly smaller leaves than do those with yellow or darker colored seeds.

### Food value of the tepary

There is considerable difference of opinion as to the relative palatability of beans and teparies. Among the Indians and Mexicans the pink bean is preferred to the tepary, as they say it has a better flavor. These people, however, make the same difference between the pink bean and the white navy beans which are shipped in from the East.

Teparies should be soaked 12 hours before cooking, during which time they swell to at least twice their original volume and more than double in weight. In this respect they markedly surpass the bean. The greater swelling and absorptive power of the tepary is probably due to its greater initial specific gravity, which is about 1.33 as compared with 1.22 for the bean. After

soaking and cooking thoroughly, the tepary is no more dense or heavy than the bean, having overcome this in its greater expansion.

A gentleman well acquainted with the customs of the Mexican and Indian users of the teparies states that these legumes are preferred to the bean and are highly prized for the preparation of light soups. At first I thought this might be due to the extraction of less solid matter, resulting in a thinner, more delicate soup. An experiment, however, was carried out whereby an equal dry weight of beans and teparies were boiled until well done. The liquors were then made up to equal volumes, well shaken, and 100 cc. of each evaporated to dryness. Contrary to expectation, the dry residue was greater for the teparies than for the beans. The residue from the teparies was much clearer, more gelatinous, and dried into a translucent, horny flake; whereas that from the beans was more friable, and dried into a somewhat brittle mass.

Under the husbandry of the Indians, this group has shown great variation. I have been able to segregate and grow more than 40 distinct agricultural varieties and I do not doubt that many more exist which I have not yet secured. In productivity, especially where the conditions are adverse, the tepary far exceeds the bean. In 9 experiments covering three years' work at Yuma, two at Tucson, and three at McNeal, the average yield of the tepary varieties has been slightly in excess of four times the average for the varieties of the kidney bean. The explanation of these greater yields on the part of the teparies, in all probability, lies in the perfect physiological adaptation of these native plants to the climatological environment in which they had their development both as wild and domesticated species. As examples of this perfect adaptation may be mentioned the following:

a) Ability to germinate quickly in the presence of a low moisture content of the soil. This enables them to take advantage of the sudden desert downpours which wet the ground and then rapidly disappear by evaporation. Teparies, which are able to germinate and come up in 3-5 days' less time than the bean, are thus able to get their leaves into the air and their roots into the soil by the aid of showers which would be wholly inadequate for securing a stand of a less specialized crop.

When tepary seeds are placed in moist soil and the soil is pressed snugly about them, they will take up enough moisture to cause the skins to wrinkle within 5 minutes. When thrown in water, they will wrinkle within 3 minutes to a condition such as is assumed by the skin of the kidney bean only after 3-6 hours of soaking (fig. 11).

b) When once the tepary has established itself in the soil, it is able to withstand protracted seasons of water famine without permanent injury. The ability to recover from the effects of

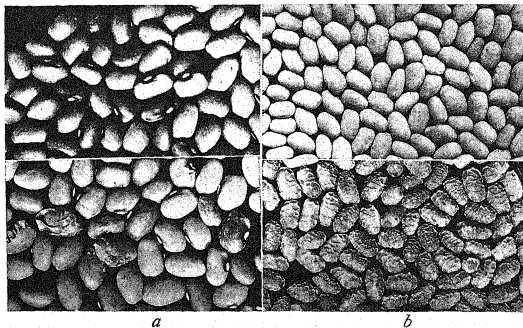


FIG. 11.—Above, beans dry (a) and teparies dry (b); below, beans after soaking in water 45 minutes (a) and teparies after soaking in water 3 minutes (b).

drought is not possessed by beans to a marked degree. This places them at a great disadvantage in a competition with the tepary under such conditions as are frequently met in the practice of dry farming.

c) The tepary is inured to the greatest extremes of our summer temperatures and will bloom and set seed during any month from May to November. On the other hand, when the blooming period of beans happens to fall within the season of extreme heat, the buds will for the most part either abort before blooming or else the flowers will fall without setting pods. For these reasons the tepary is a

more sure and dependable crop, often giving fair returns when beans are a total failure (fig. 1). As a dry land crop in the south-central plains and Rocky Mountain regions the tepary will in the near future in all probability find a place of considerable economic importance.

ARIZONA AGRICULTURAL EXPERIMENT STATION  
TUCSON, ARIZONA

A COMPARISON OF THE AMERICAN BROWN-ROT  
FUNGUS WITH *SCLEROTINIA FRUCTIGENA*  
AND *S. CINEREA* OF EUROPE

W. A. MATHENY

(WITH SIX FIGURES)

Is the American brown-rot fungus (*Sclerotinia fructigena*) of stone fruits identical with the fungus bearing the same name and occurring throughout Europe, but there found exclusively upon pome fruits? That *S. fructigena* should occur in Europe only on pome fruits and in this country only on stone fruits appears unusual and to many improbable. If *S. cinerea* is the fungus attacking stone fruits in Europe, as all the European writers maintain, then the suggestion seems pertinent that our brown rot of stone fruits is caused by the same fungus. In fact, ADERHOLD and RUHLAND (5) assert that our species is *S. cinerea*. They came to this conclusion after an examination of a few preserved apothecia sent them by NORTON, and in the absence of a more extended study their evidence appears inconclusive.

At the suggestion of Professor C. F. HODGE of Clark University, the writer has undertaken a comparative study of these two species. At first it was intended merely as a study in variation, but it soon of necessity became a problem in taxonomy. Our final interest was to determine as nearly as possible which one of the above species agrees more nearly with the rot of our stone fruits.

Historical sketch

The history of *Sclerotinia fructigena* dates back to 1796, when PERSOON (17) gave the name *Torula fructigena* to a fungus which he found on the decayed fruit of *Prunus domestica*, *Amygdalus persica*, and *Pyrus*. This same name was retained by other writers, notably FÜCKEL (12), ALBERTINI and SCHWEINIZ (6), SACCARDO (23), and RABENHORST (21). In 1801 PERSOON (18) changed it to *Monilia fructigena*, a name by which it is generally

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known today. KUNZE and SCHMIDT (14) a little later referred it to *Oidium fructigenum*, under which name it received attention from many writers, among the first of whom should be mentioned EHRENBURG (10), COOKE (8), DUBY (9), and FRIES (11). In 1822 PERSOON (19) renamed this fungus *Acrosporium fructigenum*, a classification which was never accepted by other writers. WALLROTHIO (29) placed it in the genus *Oospora*, calling it both *O. candida* and *O. fructigena*, while VON THÜMEN (27) first classed it as *Oidium Wallrothii*, but later (28) changed it to *O. fructigenum*. The first writers to attach any economic importance to this fungus were VON THÜMEN (28) and HALLIER (13). SCHRÖTER (25) in 1893, being confident of its ascomycetous nature, placed it in the genus *Sclerotinia*. This classification was confirmed in Europe in 1904 by ADERHOLD (1). NORTON (16) had first discovered apothecia in 1902.

*Sclerotinia cinerea* was first described as *Monilia cinerea* by BONORDEN (7) in 1851. He noted it forming small, gray, sometimes brownish conidial tufts on fruit. This species has many times been confused with *S. fructigena*, in fact many of the descriptions given the one could apply equally well to the other. SACCARDO (23) in 1886 recognized *Monilia cinerea*, while SCHRÖTER transferred it to the genus *Sclerotinia* along with *M. fructigena*. WORONIN (30) in his excellent study published in 1900 established beyond a doubt the fact that the species are distinct. This opinion is held by SORAUER (26).

#### Difference between *S. fructigena* and *S. cinerea*

Several points of difference between these two species have been cited by ADERHOLD (2, 3, 4), WORONIN (30), and others. Of these, the most striking are as follows:

1. The conidia of *S. fructigena* are always larger than those of *S. cinerea*.
2. There is a difference in shape of the conidia, those of the former having an elongated ellipsoidal form, while those of the latter are more rounded.
3. The conidial tufts of *S. fructigena* are light brownish-yellow or ochraceous and are always large, while those of *S. cinerea* are ash-gray

and always small. It is noted that the conidial tufts of the former often grow together, exhibiting a smooth upper surface. This does not occur with the latter species.

4. *S. fructigena* occurs on pome fruits, while *S. cinerea* occurs on stone fruits.

5. The ascospores of *S. fructigena* are sharply pointed at the ends, while those of *S. cinerea* are rounded on the ends. The former are without oil droplets, the latter possess them.

### Observations

Our observations in this study are limited to behavior on fruit and in pure culture, size of conidia, and size of asci and ascospores. No apothecia could be obtained from Europe; however, the measurements made by ADERHOLD and RUHLAND were available.

In the beginning, letters requesting pure cultures or mummied fruits bearing both species were sent to different parts of Europe. Pure cultures of *S. cinerea* and *S. fructigena* were twice sent me by Dr. J. WESTERDIJK of the Association internationale des botanistes, Bureau pour la distribution de cultures de moisissures, Amsterdam. Mummied fruits bearing *S. fructigena* were sent me by the following persons: Dr. LUIGI MONTEMARTINI of the University of Pavia; Professor Dr. C. WEHMER of Hannover; Professor Dr. PAUL SORAUER of Berlin; GY DE ISTVANFFI of Budapest; and Professor Dr. G. LUSTNER of Geisenheim. Similar fruits were also obtained from the government experiment stations at Geisenheim and Dahlem. I also received a box of mummied fruits from the Department of Agriculture of New South Wales.

Among the fruits sent me by Dr. LUSTNER were some mummied plums bearing *Sclerotinia cinerea*. Its outward appearance was so strikingly different from that of *S. fructigena* that its recognition was a matter of first glance. Both species were easily isolated on other fruits and then in pure cultures. This plan gave an easy and practical method of observing the forms as they grew side by side, many times on the same pear or apple and oftentimes in the same petri dish.

BEHAVIOR ON FRUIT.—Quinces taken from the same tree, and subjected to the same external conditions, were inoculated with



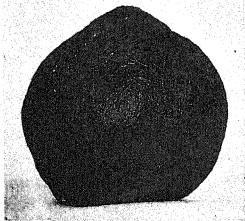
*A**B*

FIG. 1.—*A*, quince bearing the European *S. fructigena*; conidia taken from a mummied fruit obtained from Dr. P. SORAUER of Berlin; *B*, quince taken from the same tree as *A*, but inoculated with local *Sclerotinia* spores; quinces inoculated with the European *S. cinerea* behaved in a similar way.

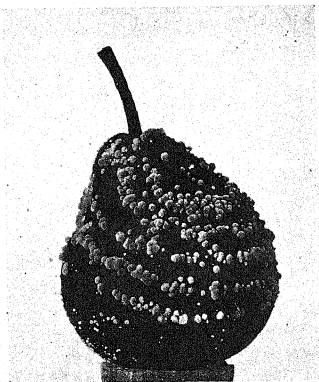


FIG. 2.—*S. fructigena* on a pear; inoculated from a fruit obtained from Dr. C. WEHMER of Hannover.

conidia of *S. fructigena* and of *S. cinerea* which were sent from Europe, and with conidia from *Sclerotinia*-infected plums growing in Worcester. The growth of *S. fructigena* was very slow, however, producing in two weeks large, yellowish conidial tufts which arrange themselves in rings (fig. 1, *A*). The quinces inoculated with *S. cinerea* and the local *Sclerotinia* behaved in exactly the same way, both turning the fruit brown but producing no conidia. In different varieties of apples, 25 experiments gave exactly similar

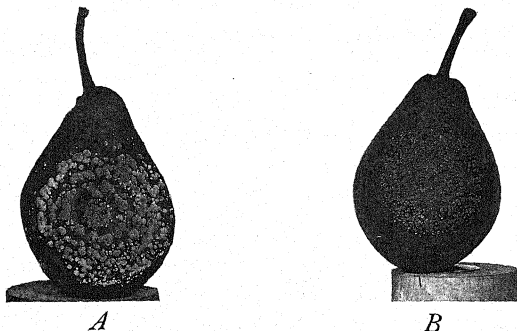


FIG. 3.—*A*, *S. fructigena* on a pear; inoculated from fruit obtained from Dr. LUIGI MONTMARTINI of Pavia; *B*, local *Sclerotinia* on a pear exactly similar to *A*; this pear was inoculated 10 days after *A*, and both photographed the same day, 13 days after the inoculation of *A*.

results as those obtained with the quinces, that is, large, yellowish conidial tufts from the *S. fructigena*, and no conidia at all from the *S. cinerea* and the local *Sclerotinia*. If the apples were cut in quarters, and the conidia placed directly on the exposed flesh of the fruit, an abundant growth of spores was obtained. Quartered apples inoculated in this manner with the spores of *S. fructigena* always gave large, yellowish tufts of conidia. When *S. cinerea* was used, the conidia appeared in small ash-gray tufts entirely unlike those of *S. fructigena*. The local *Sclerotinia* spores produce

conidia a little sooner than those of *S. cinerea*, but in other respects their resemblance is very close.

On pears, 100 experiments were tried. With the *S. fructigena* inoculations the results were uniformly the same as on the quince and apple. The growth was much slower than that of the local *Sclerotinia* on similar fruit, but the usual large, yellowish conidial tufts were produced (fig. 2).

The European *S. fructigena* and the local *Sclerotinia* were grown many times on the same pear (figs. 3 and 4), and the results were no different from those obtained when they were grown separately.

BEHAVIOR IN PURE CULTURE.—Pure cultures of the European *Sclerotinia fructigena* and of the local brown-rot fungus have been grown side by side during the past year (fig. 5). Hundreds of cultures of each have been maintained on various media: plum agar, bread agar, beet decoction, apple gelatin, etc. It was thought that should these fungi be identical, a similarity in cultural growth might appear in some of these media. No evidence of any such similarity has so far been observed. Cultural characteristics of each are as distinctly different today as they were one year ago when this experiment was started.

#### Comparison of the conidia of *S. fructigena*, *S. cinerea*, and local *Sclerotinia*

In the accompanying table are arranged the comparative sizes of the conidia of *S. fructigena* and *S. cinerea* as they have been given by different writers.

AUTHOR	CONIDIA	
	<i>S. fructigena</i>	<i>S. cinerea</i>
Saccardo (23).....	25×10-12	15-17×10-12
Lindau (15).....	20-24×12-14	12-13×9-10
Woronin.....	20.9 × 12.1 to 24.5 × 13.2	12.1 × 8.8 to 13.2 × 9.9
Schröter.....	18-24×10-12	15-18×10-12
Aderhold and Ruhland	25×13	13.8 × 9.9

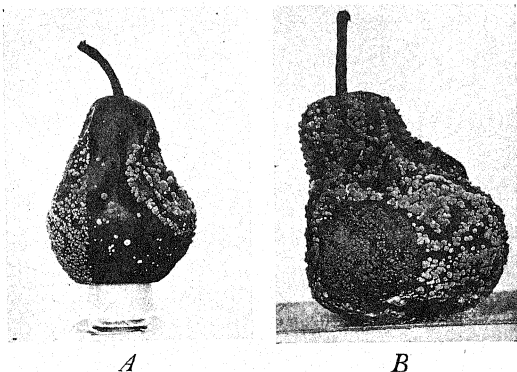


FIG. 4.—*A*, local *Sclerotinia* and European *S. fructigena* on the same pear; *B*, local *Sclerotinia* on a pear surrounded by *S. fructigena* taken from fruit sent by Dr. GY DE ISTVANEFFI of Budapest.

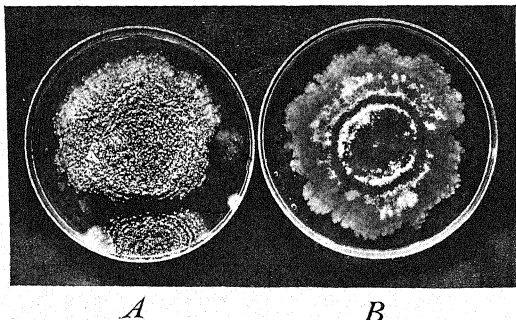


FIG. 5.—*A*, local *Sclerotinia* from plums on 1 per cent sugar, 1 per cent malic acid, and 6 per cent gelatin medium; *B*, *S. fructigena* from Europe on the same medium; both plates had the same external conditions; in both plates the gelatin is liquefied in *B* sooner and to a greater extent.

Before attempting to give the size of the conidia of the American brown-rot fungus, it was considered necessary to make a series of comprehensive measurements of the spores taken from fruit from different parts of the country. Such measurements were made. The method of this work was that of the ordinary laboratory procedure. An ocular micrometer having a scale value of  $3.6 \mu$  was used. Unconscious selection was avoided by measuring all the spores that arranged themselves in bunches on the slide, or always measuring the one nearest the right side of the eyepiece scale. Care was taken to allow the spores to become fully turgid in the water before the measurements were started.

The results obtained in these measurements are of considerable interest from another point of view. WORONIN and POLLOCK (20) have suggested that these conidia vary in size to a considerable degree according to the host or culture. Others suggest that light and darkness also cause a variation in size. In none of these instances, however, is the method of measurement mentioned or the number of conidia measured. In the results obtained in this study no such variation was ever in evidence. On the other hand, when all the measurements were done and the figures computed for a mean, variation in size of conidia was found to be practically nothing.

#### SPORE MEASUREMENTS

(In each case the upper row of figures represent microns, and the lower row the number of spores of that size.)

SPORES FROM MUMMIED PLUMS SENT BY J. W. ADAMS, FRANKLIN, IND.

No. spores measured	Length										Mean
	11.5 3	12.2 4	13 13	13.7 20	14.4 50	15.1 27	15.8 19	16.6 6	17.2 1	18 2	
145	Width										9.4 $\mu$
	7.2 4	7.9 23	8.6 31	9.4 47	10.2 19	10.8 11	11.5 7	12.2 3	.....	.....	

## SPORES FROM MUMMIED PLUMS SENT BY F. K. HACKETT, LACONIA, N.H.

No. spores measured	Length											Mean
107	13 4	13.7 7	14.4 12	15.1 31	15.8 14	16.6 14	17.2 7	18 13	18.7 3	19.4 1	20.1 1	15.1 $\mu$
	Width											
	8.6 8	9.4 8	10.2 21	10.8 34	11.5 23	12.2 11	13 2	.....	.....	.....	.....	10.8 $\mu$

## SPORES FROM MUMMIED APRICOTS SENT BY E. H. SMITH, BERKELEY, CAL.

No. spores measured	Length												Mean
146	12.2 2	13 7	13.7 12	14.4 26	15.1 30	15.8 21	16.6 15	17.2 11	18 7	18.7 5	19.4 6	20.1 4	15.1 $\mu$
	Width												
	8.6 12	9.4 11	10.2 29	10.8 43	11.5 26	12.2 15	13 5	14.4 3	15.1 2	.....	.....	.....	10.8 $\mu$

## LOCAL SCLEROTINIA ON CHERRIES

No. spores measured	Length											Mean
133	11.5 2	12.2 7	13 13	13.7 15	14.4 28	15.1 36	15.8 14	16.6 6	17.2 6	18 5	19.4 1	15.1 $\mu$
	Width											
	7.9 17	8.6 34	9.4 34	10.2 28	10.8 14	11.5 3	12.2 3	.....	.....	.....	.....	9 $\mu$

## LOCAL SCLEROTINIA ON APRICOT

No. spores measured	Length											Mean
166	11.5 2	12.2 11	13 18	13.7 27	14.4 41	15.1 21	15.8 17	16.6 4	17.2 8	18 2	18.7 3	14.4 $\mu$
	Width											
	7.2 4	7.9 11	8.6 37	9.4 49	10.2 28	10.8 14	11.5 16	12.2 7	.....	.....	.....	9.4 $\mu$

## LOCAL SCLEROTINIA ON BALDWIN APPLE

No. spores measured	Length												Mean
245	12.2 5	13 14	13.7 41	14.4 62	15.1 32	15.8 21	16.6 13	17.2 17	18 16	18.7 13	19.4 9	20.1 2	14.4 $\mu$
	Width												
	7.9 11	8.6 24	9.4 41	10.2 62	10.8 42	11.5 39	12.2 17	13 9	.....	.....	.....	.....	10.2 $\mu$

CONIDIA OF *S. fructigena*, FROM FRUIT SENT BY DR. GY DE ISTVANFFI, BUDAPEST

No. spores measured	Length										Mean
201	18 14	18.7 21	19.4 27	20.2 30	20.8 35	21.6 49	22.3 13	23 6	23.7 4	24.4 2	21.6 $\mu$
	Width										
	8.6 7	9.4 18	10.2 50	10.8 62	11.5 31	12.2 19	13 8	13.7 3	14.4 3	.....	10.8 $\mu$

CONIDIA OF *S. fructigena*, FROM AN AMERICAN QUINCE WHICH HAD BEEN INOCULATED WITH CONIDIA SENT BY THE GOVERNMENT EXPERIMENT STATION AT DAHLEM (BERLIN)

No. spores measured	Length														Mean
202	.... 1	17.2 4	18 16	18.7 7	19.4 18	20.2 22	20.8 23	21.6 64	22.3 24	23 12	23.7 5	24.4 3	25.1 2	25.8 1	21.6 $\mu$
	Width														
	8.6 10	9.4 11	10.2 19	10.8 67	11.5 21	12.2 25	13 15	13.7 19	14.4 11	15.1 1	15.8 2	16.6 1	.....	.....	10.8 $\mu$

CONIDIA OF *S. fructigena*, ON A PEAR

No. spores measured	Length															Mean
303	18 4	18.7 4	19.4 10	20.2 16	20.8 24	21.6 47	22.3 82	23 28	23.7 22	24.4 21	25.1 25	25.8 10	26.5 5	27.2 2	27.9 3	22.3 $\mu$
	Width															
	10.2 1	10.8 12	11.5 49	12.2 61	13 48	13.7 45	14.4 46	15.1 32	15.8 4	16.6 3	17.2 1	18 1	.... ....	.... ....	.... ....	21.2 $\mu$

CONIDIA OF *S. cinerea*, FROM FRUIT SENT BY DR. G. LUSTNER, GEISENHEIM

No. spores measured	Length										Mean
	11.5 4	12.2 15	13 24	13.7 30	14.4 44	15.1 34	15.8 18	16.6 5	17.2 5	18 2	
181	Width										
	8.6 7	9.4 14	10.2 31	10.8 49	11.5 24	12.2 25	13 13	13.7 9	14.4 9	.... ....	10.8 $\mu$

Number of American brown-rot conidia measured, 942; average size,  $14.7 \times 9.9 \mu$ .

Number of conidia of *S. cinerea* measured, 181; average size,  $14.4 \times 10.8 \mu$ .

Number of conidia of *S. fructigena* measured, 665; average size,  $22.1 \times 11.2 \mu$ .

## Asci and ascospores

Some attention has been given to the size of the asci and ascospores of *Sclerotinia fructigena* and *S. cinerea*. In the accompanying table are given the different measurements that have been made.

AUTHOR	<i>S. fructigena</i>		<i>S. cinerea</i>	
	Asci	Ascospore	Asci	Ascospore
Aderhold and Ruhland . . .	120-180 X 9-12	11-12.5 X 5.6-6.8	89.3-107.6 X 5.9-6.8	6.2-9.3 X 3.1-4.6
Reade (22) . . . . .	125-215 X 7-10	10-15 X 5-8	.....	.....
Pollock (20) . . . . .	130-179 X 9.2-11.5	11.4-14.4 X 5-7	.....	.....

The following explanation should be given of the above measurements. The asci and ascospores of *S. fructigena* measured by ADERHOLD and RUHLAND were obtained from mummied apples in their experiments at Dahlem. Their *S. cinerea* material was the preserved material sent them by NORTON. The measurements made by READE were of fresh material sent him by NORTON. POLLOCK'S measurements were of fresh material obtained in Michigan.



In order to determine what variation in size of asci and ascospores occurs in this country, a series of measurements was made of fresh material from different states. The measurements were made in the usual way. In each instance 100 ascospores and 70 asci were measured. From the resulting figures the following table is arranged.

MEASUREMENTS OF ASCI AND ASCOSPORES

Apothecia from	Host	Asci	Ascospores
New Haven, Conn. ....	Peach	144-188×6.8-10.8 Mostly 162×8.6	10.8-14.4×4.3-7.6 Mostly 12.6×5.4
Grafton, Mass. ....	Peach	136-202×7.5-11 Mostly 169×9.4	11.5-14×5-7.2 Mostly 12×5.4
Grafton, Mass. ....	Plum	144-162×7.2-10.8 Mostly 151×9.3	10-14×5-7.6 Mostly 12.2×6.1
West Boylston, Mass. ....	Peach	133-187×6.8-10 Mostly 158×8.2	10.8-15.1×5.4-7.2 Mostly 12.9×6.1
College Park, Md. ....	Peach	129-194×7.2-10.8 Mostly 162×9.3	8.6-15.1×5.7-7.9 Mostly 12.2×6.4
College Park, Md. ....	Plum	126-183×6.5-10.8 Mostly 151×9.6	8.6-14.4×5-7.2 Mostly 11.5×6.4
Madison, Wis. ....	Peach	137-190×6.5-10.8 Mostly 165×9.3	10.8-15.1×5-7.2 Mostly 13.1×6.5
Geneva, New York. ....	Peach	133-176×6.5-9.4 Mostly 160×8.4	11.5-14.4×5.7-8 Mostly 12.8×6.2
Lafayette, Ind. ....	Peach	135-192×7.2-11.1 Mostly 166×8.8	9.4-13.7×5.6-7.9 Mostly 12.2×6

GENERAL AVERAGE OF THE ABOVE MEASUREMENTS

Host	Asci	Ascospores
Peach. ....	135-190×6.9-10.5 Mostly 163×8.9	10.5-14.5×5.2-7.5 Mostly 12.5×6
Plum. ....	135-173×6.8-10.8 Mostly 151×9.4	9.3-14.2×5-7.4 Mostly 11.8×6.3

It is seen that the results here obtained correspond closely with the measurements given by POLLOCK and READE, indicating that the same species was considered in all three cases. It is further noted that these measurements correspond with those given by ADERHOLD and RUHLAND for the European *S. fructigena* and are very little larger than those given by them for their *S. laxa* which they obtained from apricots. It is to be remembered that in the case of both these species very few measurements have been

made, and that a more comprehensive study may reveal a wider variation than is here indicated.

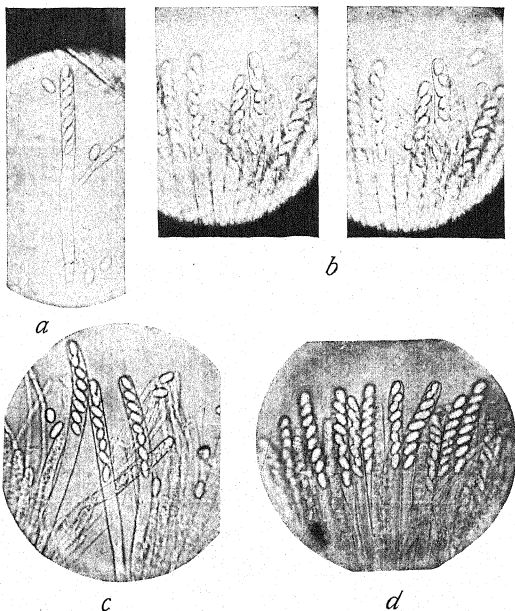


FIG. 6.—Asci and ascospores: *a*, ascus from Maryland plum; *b* and *c*, asci and ascospores from Maryland plums; *d*, from Maryland peach; all  $\times 400$ .

### Conclusions

The 300 experiments on different fruits show in every instance a wide difference between the *S. fructigena* of Europe and the local brown rot. First, they differ in the rate of growth, the former

being much slower than the latter (fig. 3). Second, the conidial tufts do not agree in size, shape, or color. The *S. cinerea* when grown on plums, pears, apples, and quinces agrees in practically every instance with the local *Sclerotinia*.

When grown in pure culture, the European *S. fructigena* never agreed with the local form (fig. 5); 300 cultures of each were made. The conidia of the former are larger than those of the latter. Those of the latter, however, agree in size with the conidia of *S. cinerea*.

While the asci and ascospores of the European *S. fructigena* and the American form apparently correspond in size, there are differences that remain distinct. The ascospores of the former are sharply pointed at each end and are free from oil droplets, while the ascospores of the latter are rounded at the ends and possess oil droplets (fig. 6). No exception was found to this rule.

The American brown rot of stone fruits is not identical with *S. fructigena* occurring in Europe on pome fruits. It agrees more nearly with *S. cinerea* and should be referred to that species.

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## OSMOTIC PRESSURE IN POTATOES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 177

M. A. BRANNON

(WITH FOUR FIGURES)

During recent years the problems of periodicity, dormancy, and after-ripening have been investigated by numerous plant physiologists. Among the numerous questions involved, that of osmotic pressure has a prominent place. Naturally, the study of osmotic pressure in living cells affords a difficult and intricate field of research provided the direct method of measurement is employed. If an indirect method, such as the cryoscopic, be used, the principal difficulty is to secure sufficient sap, in the event that the freezing temperature is determined by the Beckmann apparatus. The potato tuber satisfies this requirement in an excellent way. This paper is intended to present some of the results obtained in the study of the osmotic pressure of the sap taken from potato tubers kept at room temperature and in ice boxes. In this brief paper, there is no intention of giving a review of literature pertaining to the subject of osmotic pressure in its relation to after-ripening. The literature on after-ripening has been completely presented by Miss ECKERSON in a recent paper.<sup>1</sup>

### Methods

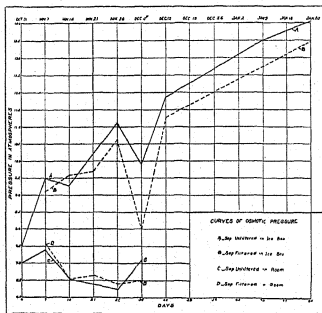
In studying osmotic pressure in potatoes, an effort was made to reduce the experimental work to a single factor and that the limiting factor, heat. Various kinds of potatoes were used, such as the Burbank, Russet Burbank, Triumph, Snowflake, and Dakota Red. The potatoes were gotten in the autumn, wrapped in paraffine paper, and placed in two collections. One was buried in dry sand or gravel and kept at room temperature of 22-25° C.

<sup>1</sup> ECKERSON, SOPHIA, A physiological and chemical study of after-ripening, BOT. GAZ. 55:286-299. 1913.

The other potatoes were placed in a dark box and inclosed in an ice chest the temperature of which was  $2-5^{\circ}$  C. At different intervals specimens were taken from each collection, washed carefully, peeled so that considerable cortex was left with the central part of the potato, and then they were broken down with a glass scraper in a porcelain dish and the sap expressed from the potato pulp. This was tested in the Beckmann apparatus, the directions given by HAMBURGER in *Osmotischer Druck und Ionenlehre* being followed.

### Procedure and results

The accompanying graphs indicate the readings at stated intervals. Graph 1 (fig. 1) shows the gradual rise of osmotic pressure in



the ice box Burbank potatoes between the beginning and the close of the experiment, October 31, 1911, to January 23, 1912. At the latter date the pressure of the potato sap measured in atmospheres was 14.051. The pressure measurements of the sap in the room potatoes in this experiment ceased on December

5, when the osmotic pressure, measured in atmospheres, reached 7.4. During the progress of this work it seemed possible that filtration might prove interesting, consequently a set of tests with the filtered sap paralleled those made with the unfiltered sap. In most cases the readings of the unfiltered were higher than those of the filtered sap.

The notable diminution in the pressure curve for December 5 was very difficult to account for at the time the reading was

made. Subsequently it appeared that it was largely due to the fact that small specimens were taken from the ice box on that date. The size of the potato is of such consequence that the potatoes should be selected for uniformity at the beginning of the experiment. What the causal agent is has not been determined, but it uniformly appeared in the various tests made with the different varieties used in these osmotic studies.

Graph 2 (fig. 2) contains a summary of the readings made in a set of experiments extending from January 22, 1912, to July 17, 1912. A pronounced lift in osmotic pressure in the sap taken from

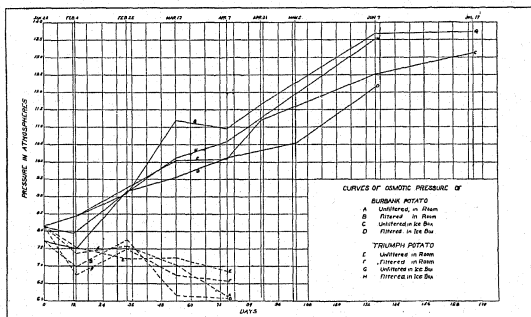


FIG. 2

the ice box potatoes is well illustrated, and the depression in the osmotic pressure in the sap taken from the room potatoes is also clearly shown. In the progress of this work there are certain interesting variations which seem to be related to the question of filtration of sap and also intimately associated with the size of the potatoes from which the sap was secured. As already noted, the curve for the unfiltered potato sap ran considerably higher on the average than that of the filtered. It was more pronounced in the case of the ice box potatoes than in those kept at room temperature. The filtered and unfiltered sap from ice box Burbank potatoes

showed a varying pressure ranging from 0.1 to 0.9 atmospheres. The Triumph ice box potatoes gave a somewhat larger variation when filtered and unfiltered sap collections were tested, one reading amounting to 1.08 atmospheres greater pressure in the unfiltered specimen.

The difference of pressure in the sap taken from a large and a small Burbank in the ice box ranged from 0.5 to 1.32 atmospheres, and in the case of sap obtained from a large and a small Triumph, kept in the ice box for 8 weeks, there were 1.69 atmospheres in favor of the larger specimen.

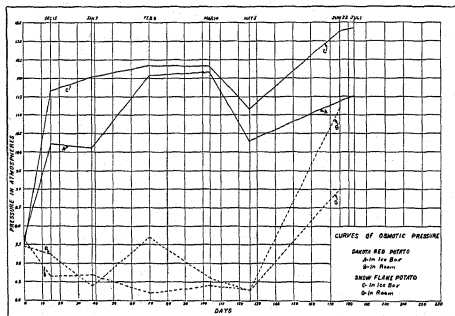


FIG. 3

The third graph (fig. 3) represents the readings which have been taken in a study of the Dakota Red, which is a very large potato, and the Snowflake which is much smaller. This set of readings extended from October 31, 1912, to July 1, 1913. Again the rapid ascent of osmotic pressure in the potatoes kept in the ice box is clearly shown, whereas the osmotic pressure of those kept at room temperature declined.

The rapid lift in the curves B and D in the room temperature potatoes is associated with the loss of water which left these specimens and moved into young potatoes that were developed between



May 5 and July 1, and a consequent concentrating of the solutes in the sap which was expressed with great difficulty from the old, wrinkled potatoes kept 8 months in dry sand at room temperature.

In the last series of readings a comparison of filtered and unfiltered sap was not made in every case, but whenever the test was conducted the unfiltered sap showed somewhat greater pressure than the filtered.

The fact that the larger potatoes gave a higher osmotic pressure than smaller ones seems to hold only between those within a given variety. At any rate, in this last set of readings the Snowflake, which is a much smaller potato than the Dakota Red, gave a higher pressure. However, it was found that a small Dakota Red gave

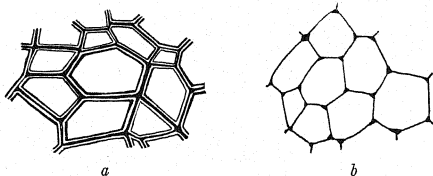


FIG. 4.—Tissue of potatoes 140 days after test began: *a*, Dakota Red potato at room temperature, with thick walls,  $\times 136$ ; *b*, ice box potato, with thin walls,  $\times 136$ .

a lower pressure than a large Dakota Red kept under the same control conditions, and likewise that a small Snowflake gave a lower pressure than a larger Snowflake when they had been kept under the same control conditions.

It was observed in macerating the potatoes used in the readings recorded in this paper that the tissues of the ice box potatoes became increasingly more brittle with the lengthening of the experiment. The supply of free starch became reduced and the proportionate amount of water seemed to be increased. The room potatoes tested at the same time in the control work were found to have an increasing toughness as the time of the experiment extended. This result was clearly related to the cell-wall structure. The accompanying drawings (fig. 4) indicate why the tissues of the ice box potatoes broke down so much more readily under the macer-

ating tool than did the tissues of those kept at room temperature. In the former, no hemicellulose remains after 90-120 days. The drawings show the situation in the tissues of the room temperature potatoes and the ice box potatoes after a test of 140 days.

The chemical reaction of the tissues taken from the ice box and room temperature potatoes indicates a greater acidity in the former than in the latter. Furthermore, it shows that the acidity is greatest in the eye region, and that it gradually diminishes until it quite disappears as the distance from the eye into the cortex increases.<sup>2</sup>

Comparative tests of osmotic pressure in sap taken from the cortical part of the potatoes and from the vascular cylinder portions indicated that there was considerable difference in the sap taken from these regions in the same potatoes. There were minor variations noted which were easily within the range of error incident to the instrument and the indirect method employed in determining the osmotic pressure.

### Conclusions

1. Heat is a limiting factor in controlling the processes which develop the substances that give rise to variation in osmotic pressure in potato sap.

2. Lowering temperature causes an increase in acidity, which in turn seems to be the controlling agent in the release of the enzymes which hydrolyze starch and hemicellulose.

3. The carbohydrates hydrolyzed furnish the energy which is used by the potato while carrying on its metabolism during cold storage.

This work was undertaken at the suggestion of Dr. WILLIAM CROCKER to whom I am greatly indebted for much valuable assistance.

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<sup>2</sup> For the acid determinations I am indebted to Dr. SOPHIA ECKERSON.

THE CASTOR BEAN PLANT AND LABORATORY AIR  
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 178

E. M. HARVEY

It is well known that the leaves of the castor bean (*Ricinus communis*) are likely to show nastic drooping after the plant has been brought into the laboratory. On account of the apparent definiteness of this response, it seemed probable that the plant would prove useful as a delicate test for certain gaseous impurities in laboratory air. It was with regard to this possibility that the studies reported below were undertaken.

Potted seedlings, grown under ordinary greenhouse conditions until they had developed 5-7 leaves, including the cotyledons, were used in the tests. The pots and soil were covered with paraffin to avoid absorption of the gases. Exposure periods were all approximately 60 hours. The methods of exposure to known concentrations of ethylene and illuminating gas were the same as described by KNIGHT and CROCKER<sup>1</sup> for these gases in their work on sweet pea seedlings. In the main, the 50-100-liter galvanized iron cans were used for exposure chambers, but, since here the plant would be in total darkness during the course of the experiment, some were exposed under bell jars provided with water seals and left in strong diffused light, in order to find what effect light would have on the sensitiveness of the response. Corresponding results, however, were obtained by the two methods. Illuminating gas tests were run parallel with those of ethylene in a manner to yield evidence whether the reaction was induced by the ethylene constituent of the illuminating gas. Ethylene occurs in the Chicago illuminating gas in the concentration of about 4 per cent, so pure ethylene was diluted with air to 4 per cent, so that, volume for volume, the illuminating gas and the ethylene-air mixture held about the same amount of ethylene. This stock mixture allowed parallel experiments to be carried out readily.

<sup>1</sup> KNIGHT, L. I., and CROCKER, W., Toxicity of smoke. BOT. GAZ. 55:337-371. 1913.

The reaction itself is very easily observed. The most obvious effect of the gas is the nastic drooping of the leaves as mentioned above. This drooping is brought about by curvatures, located either at the inner or at the outer end of the petiole or at both. If the gas be of certain concentration, the older leaves may develop abscission layers that often result in leaf fall. And again, if the gas be somewhat stronger, rapid proliferation may take place at these new leaf scars, forming small masses of tissue which usually exude sap. The most delicate response of the plant seems to be a nastic folding down of the laminae of the young leaves. However, the very young leaves that are still more or less enveloped by the bud scales seldom show any response.

Data regarding the appearance and degree of these responses were obtained by observation of the following points. Previous to the exposure, the leaves of the plant were numbered consecutively from cotyledons upward. Also the condition and direction of the plane of the leaves were noted. This consisted in an examination for abscission layers already started, the measuring of the angle made by each petiole with the stem, and the angle made by the plane of the leaf surface with the petiole. After exposure, examination was made for leaf fall, starting of abscission layers, the new angles made by the petioles with the stem and by the plane of the leaf surfaces with the petioles, and, finally, the condition of the laminae of the young leaves was noted. The amount of nastic drooping at each end of the petioles was recorded in degrees.

The results of the experiments proved that this plant has great capacity for response to low concentrations of ethylene, just as one was led to expect from its common behavior in the laboratory. The lowest concentration tried was one part ethylene to 10,000,000 of air (or 0.00001 per cent). With this amount the response was absolutely definite, as shown, for example, in one case by a nastic drooping of  $15^{\circ}$  at the inner end of the petiole for leaf no. 5; by drooping of  $30^{\circ}$  and  $15^{\circ}$  at the outer ends of the petioles for leaves nos. 5 and 6 respectively; and by a nastic folding down of laminae for leaves nos. 6 and 7. Parallel experiments with illuminating gas and ethylene, where the ethylene constituent of the former was about equal to the ethylene in the corresponding

ethylene-air mixture, gave results similar in kind and degree. This fact indicates that the ethylene of the illuminating gas is responsible for the reaction. Especially does this seem true in the light of similar results obtained by KNIGHT and CROCKER on sweet pea seedlings. The definiteness of the reaction with one part ethylene to 10,000,000 of air leads one to believe the plant

TABLE I

PLANT A EXPOSED TO 1 PART ETHYLENE TO 1,000,000 AIR; PLANT B EXPOSED TO 25 PARTS ILLUMINATING GAS TO 1,000,000 AIR (I.E., APPROXIMATELY 1 PART ETHYLENE TO 1,000,000 AIR); PLANT C THE CONTROL

Number of leaf	Plant	No response	Proliferation at leaf scar	Leaf fall	Starting of abscission layer	Nastic drooping of petiole; inner	Nastic drooping of petiole; outer	Nastic folding down of lamina of leaf
1 (cotyl.)	A	×	.....	.....	×	.....	.....	.....
	B	×	.....	.....	.....	.....	.....	.....
	C	×	.....	.....	.....	.....	.....	.....
2 (cotyl.)	A	.....	.....	×	.....	.....	.....	.....
	B	.....	.....	.....	×	.....	.....	.....
	C	.....	.....	.....	×	.....	.....	.....
3	A	×	.....	.....	.....	.....	×	.....
	B	.....	.....	.....	.....	.....	45°	.....
	C	×	.....	.....	.....	.....	.....	.....
4	A	.....	.....	.....	.....	×	×	×
	B	.....	.....	.....	.....	45°	45°	(?)
	C	×	.....	.....	.....	×	×	.....
5	A	.....	.....	.....	.....	.....	×	×
	B	.....	.....	.....	.....	×	45°	×
	C	×	.....	.....	.....	(?)	.....	.....
6	A	.....	.....	.....	.....	.....	×	×
	B	.....	.....	.....	.....	×	90°	×
	C	×	.....	.....	.....	(?)	90°	×

×× = very strong response.

capable of responding to even considerably lower concentrations than any yet tried. The least delicate of the reactions, the proliferation and exudation at leaf scars, usually appears in concentrations of about one part ethylene to 50,000 of air; while leaf fall and especially the starting of abscission layers may appear in concentrations as low as one part ethylene to 500,000 of air, or even one part ethylene to 1,000,000 of air. The nastic drooping of the youngest well developed leaves and the folding down of the laminae of the young leaves are the responses one must depend on for extremely low concentrations.

The accompanying table (table I) will serve to show the method of recording the reactions, and also how close is the parallelism between the ethylene and the illuminating gas under the conditions of concentration named above.

In conclusion it may be stated that the castor bean plant has proved capable of giving an easily observed response to extremely small amounts of ethylene; and on account of this fact the plant seems particularly useful for the detection of harmful gaseous impurities<sup>2</sup> in the air of laboratories and greenhouses.

The writer gratefully acknowledges the many valuable suggestions of Dr. WILLIAM CROCKER.

UNIVERSITY OF CHICAGO

<sup>2</sup> It would not be of value for such gaseous impurities as are not active in inducing this response, but ethylene, though in small amounts, is probably the most harmful and generally present of any laboratory air impurity.

# CURRENT LITERATURE

## BOOK REVIEWS

### The Bonn textbook<sup>1</sup>

For nearly twenty years the *Bonn textbook of botany* has been the most prominent text of college grade in the world, having enjoyed a wider circulation and having been translated into more languages than any of its competitors. The first edition appeared in 1894, STRASBURGER writing the morphology, NOLL the physiology, SCHENCK the cryptogams, and SCHIMPER the phanerogams. Of the four original authors, only SCHENCK remains, but they had already prepared five editions together, thoroughly establishing the book, before SCHIMPER died, a victim of his own restless researches. His place was taken by KARSTEN, who has not only maintained the high standard of his predecessor but has improved this part of the work with each successive edition. NOLL wrote the physiology for the first nine editions, and then died suddenly, in the prime of his physical and mental vigor. JOST took his place, and from this time physics and chemistry have been increasingly conspicuous in this portion of the work.

The heaviest loss of all was sustained in the death of STRASBURGER, who had been the dominating personality of the book and who had prepared the morphology for eleven editions. In the twelfth edition, dated April 1913, FITTING, who succeeded STRASBURGER at Bonn, has written the morphology. There is considerable change in arrangement, some change in subject-matter, and some change in wording, even when there has been no change in ideas. An admiring pupil and personal friend of the master-botanist of his time must be excused if he fails to find an improvement in this section of the book.

SCHENCK, the only survivor of the founders, has been throughout a reliable, consistent writer, steadily improving his contribution and refusing to be swayed by temporary flurries in his subject.

The excellent illustrations have always been a feature of Bonn texts. Some new figures have been added, and for the first time all figures have been credited to their respective authors.—CHARLES J. CHAMBERLAIN.

### Goebel's Organography

A second edition of this work<sup>2</sup> is appearing and the first volume is already before us. The revision has been thorough, some features of the first edition

<sup>1</sup> FITTING, H., SCHENCK, H., JOST, L., und KARSTEN, G., *Lehrbuch der Botanik für Hochschulen*. 12th ed. 8vo. pp. viii+620. figs. 782. Jena: Gustav Fischer. 1913.

<sup>2</sup> GOEBEL, KARL, *Organographie der Pflanzen*. Erster Teil, *Allgemeine Organographie*. 8vo. pp. x+513. figs. 513. Jena: Gustav Fischer. 1913. *M* 16; bound *M* 17.

having been abandoned and much new material with numerous figures having been added. There is a change of view in regard to leaf arrangement, and alternation of generations receives some attention. Naturally the relation between form and function, the inheritance of malformations, and the significance of juvenile stages are of interest to all botanists, and whether one agrees with the author or not, GOEBEL's account is stimulating and should put a needful restraint upon those who would blindly assign every such phenomenon to heredity and recapitulation.

The intimation, in the preface, that botanists have left the older fields of morphology needs a word of comment, for there are still some of us who feel as much interest in the problems of evolution, heredity, and phylogeny, as in seeing "the wheels go round." We welcome this book as a wholesome check. The morphologist whose principal interest is in phylogeny needs it, just as the experimental morphologist, whose principal interest is in something other than phylogeny, needs to know more about the structure and development of the objects upon which he is experimenting.

The second volume, dealing with special organography and containing the index, will be awaited with interest.—CHARLES J. CHAMBERLAIN.

## NOTES FOR STUDENTS

**Osmotic pressures.**—RENNER<sup>3</sup> has rendered a very important service to plant physiology by summarizing and subjecting to a critical analysis the literature of recent years dealing with the methods of calculating the osmotic pressure of solutions. All methods heretofore used by physiologists have been based on the assumption that an osmotic pressure of 22.4 atmospheres at 0° C. produces a freezing temperature of -1.85° C. But this depression of the freezing point, according to the physicists, is brought about when a mole of a non-electrolyte is dissolved in 1000 cc. of water; whereas physiologists have always made up their solutions as weight per cent solutions, or as molecular solutions in which enough water is used to make 1000 cc. of solution, but still assuming that the osmotic pressure of these more concentrated solutions is 22.4 atmospheres for undissociated salts. The discussion centers about the work of MORSE, FRAZER, and their co-workers, whose exact determinations have now shown clearly that such solutions must be made up in weight normal instead of volume normal concentrations in order to yield osmotic pressures agreeing with the gas laws. RENNER shows from calculations based upon the determinations of MORSE that there is a very close relation between depression of the freezing point and osmotic pressure. He claims indeed that all the disagreements in the literature between the results of the cryoscopic and plasmolytic determinations of osmotic pressure are traceable to the fact that the

<sup>3</sup> RENNER, O., Über die Berechnung des osmotischen Druckes. Biol. Centralbl. 32:486-504. 1912.



freezing point method uses weight normal, whereas the plasmolytic method uses volume normal solutions. He shows conclusively in his discussion that it is the plasmolytic method which has been in error.

The large error of the old method is illustrated by the cryoscopic measurement of a volume normal cane sugar solution. The depression of the freezing point is  $-2.66^{\circ}\text{C}$ ., which indicates an osmotic pressure of 32 atmospheres instead of 22.4 atmospheres. The excess pressure is due to the sugar being dissolved in less than 1000 cc. of water. A comparison of the pressures for 67 per cent cane sugar and 18.5 per cent NaCl as calculated from the volume normal and weight normal concentrations is very illuminating also. The NaCl solution contains 3.6 moles of salt, 50 per cent ionized, in a liter of solution; while the sugar solution contains only 2.6 moles, with no ionizing, per liter. The NaCl should develop a much higher pressure according to the plasmolytic method of estimating pressures; but adopting the method of MORSE, now shown to be correct, which demands equal quantities of solvent, we find that the NaCl is dissolved in 928 cc., the sugar in only 439 cc. of water. The relations of the two solutions are seen to be actually the reverse of what the plasmolytic method indicates. Owing to the dissociation of the NaCl, the solutions are really nearly isosmotic. These examples serve to show the unreliability of the plasmolytic determinations with volume normal solutions, and the necessity, despite their inconvenience, of using weight normal solutions in the future.

It is unfortunate that the physical chemists have not determined the osmotic pressure of saturated solutions by the cryoscopic method. While RENNER makes clear that at the concentrations thus far measured the freezing point determinations will give the correct osmotic pressure, it remains to be demonstrated that this holds for all concentrations whatsoever. The direct determinations by Lord BERKELEY and HARTLEY for concentrated sugar solutions exceed the measurements called for even by the MORSE-FRAZER method. At present, the reviewer believes that these direct measurements, recently confirmed by TROUTON, are the most trustworthy determinations we have for concentrated solutions. If the cryoscopic methods should yield results in agreement with the direct measurements at these same concentrations, and with saturated solutions of various salts, its universal application could be admitted. If it does not, then still further correction of the formula for calculating the osmotic pressure of solutions must be made. Every physiologist interested in the determination of osmotic pressures should by all means read RENNER's excellent discussion.—CHARLES A. SHULL.

**Paleobotanical notes.**—ARBER<sup>4</sup> has described 44 species of plants from a coal-field in Gloucestershire, none of which are new to Great Britain. The

<sup>4</sup> ARBER, E. A. NEWELL, On the fossil flora of the Forest of Dean coal-field (Gloucestershire), and the relationships of the coal-fields of the west of England and South Wales. *Phil. Trans. Roy. Soc. London B* 202:233-281. *pls. 17-13*. 1912.

contribution deals chiefly with stratigraphy, but a fact of botanical interest is that several species, including *Annularia galioides*, *Sphenophyllum majus*, *Lepidodendrom dichotomum*, and three species of *Sigillaria*, were found in a higher horizon (Upper Coal Measures) than known before in Great Britain.

MISS HOLDEN<sup>5</sup> has begun a series of contributions dealing with the anatomy of mesozoic conifers, the first one giving the results of a study of some Jurassic material from Yorkshire. The collection proved to contain some typically abietineous woods and some typically araucarian woods, but most of the woods were intermediate between the two. It is concluded that the character of these transitional woods corroborates the view that the Abietineae are the oldest conifers and that the araucarians have been derived from them. A remarkably strong statement is the following: "Comparative examination of living and fossil forms leads to the rejection of all criteria except cellulose bars of Sanio as an infallible test for tribal affinities."

MISS HOLDEN<sup>6</sup> has investigated a collection of lignite from some Middle Cretaceous beds of New Jersey. Most of the plants belonged to *Cupressinoxylon*, *Araucarioxylon*, and *Brachyoxylon*, and will be described later. In the present paper three types of *Pityoxylon* are described as new species: *Pinus protosclerophyllis*, *Pityoxylon foliosum*, and *P. anomalum*. The first mentioned is probably the earliest form with all the characters of a modern hard pine, and the occurrence of such a pine as early as the Middle Cretaceous is thought to argue strongly for the great antiquity of the genus *Pinus*. *Pityoxylon foliosum* is probably the wood of *Prepinus*, all the leaves being borne directly on the main axis, and combining characters that are now separated in hard and soft pines. *P. anomalum* has a woody structure like that of *Prepinus*, but all the leaves are borne on short shoots.

THOMAS<sup>7</sup> has described a leaf from the Jurassic of Yorkshire which he thinks represents a new genus (*Eretmophyllum*) of Ginkgoales. The leaves differ from those of *Ginkgo* in being oblanceolate or linear, but approach those of *Ginkgodium* in outline; the leaves of the latter, however, are shorter and comparatively broader and are often deeply divided at apex. The most important distinction, however, is found in the venation.

SEWARD<sup>8</sup> has described some dicotyledonous leaves from the coal measures of Assam, which are probably Tertiary, although there are claims that they

<sup>5</sup> HOLDEN, RUTH, Contributions to the anatomy of mesozoic conifers. I. Jurassic coniferous woods from Yorkshire. Ann. Botany 27:533-545. pls. 39, 40. 1913.

<sup>6</sup> ———, Cretaceous Pityoxyla from Cliffwood, New Jersey. Proc. Amer. Acad. 48:609-623. pls. 4. 1913.

<sup>7</sup> THOMAS, H. HAMSHAW, On some new and rare Jurassic plants from Yorkshire; *Eretmophyllum*, a new genus of Ginkgoalian leaf. Proc. Cambridge Phil. Soc. 17: 256-262. pls. 6, 7. 1913.

<sup>8</sup> SEWARD, A. C., Dicotyledonous leaves from the coal measures of Assam. Records Geol. Survey India 42:93-101. pls. 17, 18. 1913.

belong to the Cretaceous. Of course in the absence of fruits and seeds the determinations are not altogether satisfactory. In any event, the families thought to be represented, each by one to three species, are Magnoliaceae, Anonaceae, Fagaceae, Myristicaceae, Lythraceae, Moraceae, Rubiaceae, Guttiferae, Burseraceae, Anacardiaceae, Ericaceae, Dipterocarpaceae, and Ternstroemiaceae. If one can draw any conclusion from this showing, it is that the dicotyledonous series was represented practically throughout its present extent.—J. M. C.

**Morphology of Calycularia.**—CAMPBELL<sup>9</sup> has studied the rare Javanese liverwort *Calycularia radiculosa*, found in small quantities near Tjibodas. The plant is strictly dioicous, the males being decidedly smaller than the females. It perhaps more nearly resembles *Symphlogyna* than *Pallavicinia* (*Blyttia*), with which a comparison is made. The endophytic fungus common to so many liverworts is conspicuous. Two forms of apical cells are present: one is the cylindric-lenticular type found in *Pellia epiphylla*; the other is the cuneate type common to Marchantiales, *Sphaerocarpus*, and most Anthocerotales.

Branching of *Calycularia* he says, "seems to be a true dichotomy, but whether one of the branches retains the original apical cell or whether two new apical cells are developed was not investigated." In order to have true dichotomy, the original apical cell by equal division must give rise to two new apical cells. But if, on the other hand, the original apical cell is retained by one branch, the apical cell of the other branch must be formed from a segment of the original apical cell, and we have, not true dichotomy, but apparent dichotomy. Of course in most cases, whether we have true or simulated dichotomy makes little difference in the appearance of the adult plant.

The earliest stages of the antheridium were not studied, but apparently it develops like that of the majority of Jungermanniales. In the ultimate division of the spermatogenous cells a wall is formed. No trace of a "Nebenkörper" or "accessory body" was found. Archegonia are grouped on a rudimentary receptacle. The number of neck cells is various, the highest number found being eight. Occasionally a binucleate neck canal cell was found, showing that the neck is being shortened. In one archegonium neck canal cells were enlarged and closely resembled eggs. This seeming reversion to a more primitive type of archegonium seems to be widespread in bryophytes.

Early stages in embryogeny were not studied. The capsule has a relatively thick wall. The foot is the mushroom-anchor shape so common in liverworts. The spore mother cells are deeply lobed, as is characteristic of practically all Jungermanniales. The "quadripolar" spindle of FARMER was

<sup>9</sup> CAMPBELL, DOUGLAS HOUGHTON, The morphology and systematic position of *Calycularia radiculosa* Steph. Leland Stanford Junior Univ. Publ., Univ. Series, Dudley Mem. Vol. pp. 43-61. figs. 12. 1913.

noted, but occasionally a conspicuous bipolar spindle was seen. *Calycularia* therefore seems to be a suitable form for settling the FARMER-MOORE controversy, and it is greatly to be regretted that CAMPBELL's material is so scanty. He thinks that *Calycularia radiculosa* should be made the type of a new genus intermediate between *Mörkia* and forms like *Makinoa* or *Pellia*.—W. J. G. LAND.

**Embryo sac of *Aglaonema*.**—CAMPBELL<sup>20</sup> has published a further study of the embryo sac and embryo of *Aglaonema*, the species investigated being *A. simplex* and *A. modestum*. Among the results are the following: the primary sporogenous cell develops the embryo sac directly; the first divisions in the embryo sac result in four free nuclei arranged in pairs, and only one of the micropylar nuclei divides, producing the synergids, the other without division becoming the egg nucleus; there is no nuclear fusion preceding endosperm formation and there are no definite antipodal cells; no evidence of fertilization was seen; the sac becomes filled with endosperm tissue; in embryo-formation the synergids remain intact, "and it sometimes looks as if they also contributed to the tissues of the embryo"; the embryo finally completely fills the sac, the body regions being differentiated at a late stage in the development.—J. M. C.

**Sex in *Onoclea*.**—Miss WUIST<sup>21</sup> has used *Onoclea Struthiopteris* in an investigation to determine whether the sex of the dioecious gametophytes is predetermined in the spore. Soil and solution cultures were employed, also different intensities of insolation. The work has extended through several years, so that the results are well established, the fundamental one being that the sex of the gametophyte is not predetermined in the spore. It was shown that the gametophyte is either monoecious or apparently dioecious according to its age and environment; for example, in younger cultures in soil 5 per cent of all the gametophytes were monoecious; in older cultures 15 per cent were monoecious. A striking result was that 90 per cent of the gametophytes which originally bore archegonia were induced later, by "favorable conditions of nutrition," to produce antheridia; while 5 per cent of the gametophytes which originally bore antheridia were induced later to produce archegonia. The "male tendency" appeared to be latent in all parts of the apparently female gametophyte. The effect of various cultures and the incidental responses of various kinds are very suggestive.—J. M. C.

<sup>20</sup> CAMPBELL, D. H., The embryo sac of *Aglaonema*. Scottish Bot. Review 1:110-115. pls. 1-4. 1912.

<sup>21</sup> WUIST, ELIZABETH DOROTHY, Sex and development of the gametophyte of *Onoclea Struthiopteris*. Physiol. Researches 1:93-132. figs. 15. 1913.

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BOTANICAL PHENOMENA AND THE PROBLEM OF  
RECENT COASTAL SUBSIDENCE<sup>1</sup>

DOUGLAS WILSON JOHNSON

(WITH NINE FIGURES)

Fifty-five years ago GEORGE H. COOK<sup>2</sup> published his important paper on the subsidence of the land along the coasts of New Jersey and Long Island, in which he cited much evidence to prove that these coasts were gradually sinking at a rate of more than half a meter per century. Before this time a number of writers had called attention to certain phenomena which seemed to them to indicate a progressive subsidence of the coast; and since the publication of Cook's article, many reports upon this interesting subject have appeared. A few of these authors have argued in favor of the recent elevation of certain parts of the coast; a few others have maintained that the evidence of recent changes of level were not convincing; but by far the greater number have supported the theory of recent subsidence, and have described indications of a sinking of the land for almost all parts of the coast between Prince Edward Island and Florida. It is today generally accepted as a well established fact that the Atlantic coast of North America is gradually subsiding, at a rate which is variously estimated from 20 to 75 cm. per century.

<sup>1</sup> The substance of a portion of this paper formed part of an article published in the *Annales de Géographie*, May 1912, under the title "Fixité de la côte Atlantique de l'Amérique du Nord."

<sup>2</sup> COOK, G. H., On a subsidence of the land on the seacoast of New Jersey and Long Island. *Amer. Jour. Sci.* II. 24:341-354. 1857.

The supposed changes of level of the Atlantic coast have interested me for several years. A study of the form of Nantasket Beach, near Boston, showed that this portion of the coast could not have subsided more than a meter during the last 1000 or 2000 years.<sup>3</sup> An examination of certain shore line changes produced at Scituate, also near Boston, by the great storm of 1898, showed that all the appearances of a subsidence of the coast could be produced by an increased height of high tide resulting from a change in the form of the shore line.<sup>4</sup> Finally, aided by a grant from the Shaler Memorial Fund of Harvard University, by the excellent services of my three assistants, Messrs. D. C. BARTON, J. K. WRIGHT, and G. B. REED, and by the cordial cooperation of a large number of geologists, engineers, and officials of state and national surveys, I have been able to study the most important localities on the Atlantic coast from the northern side of Prince Edward Island to the Florida Keys, as well as a number of places on the coasts of Sweden, England, and Holland. The results of these studies seem to me to justify, for the Atlantic coast of North America, the following conclusions: (1) There can have been no long-continued progressive subsidence of this coast at a rate of 20 cm. or more per century, during the last few thousand years. The coast has remained at least comparatively stable throughout this period. (2) The coast cannot have subsided as much as 30 cm. in the last century. (3) There is no satisfactory evidence of any subsidence whatever during the last few thousand years.

In the present paper no attempt is made to consider all phases of the interesting problem of recent coastal subsidence; attention is here directed exclusively to the consideration of some of the botanical phenomena supposed to prove such a subsidence. If apology is needed for my venturing to discuss botanical phases of the question, and in a botanical journal, my plea is that I am a firm believer in what has been aptly termed cross-fertilization of the sciences. The study of a complex problem has forced me to

<sup>3</sup> JOHNSON, D. W., and REED, W. G., The form of Nantasket Beach. *Jour. Geol.* 18:162-189. 1910.

<sup>4</sup> JOHNSON, D. W., The supposed recent subsidence of the Massachusetts and New Jersey coasts. *Science N.S.* 32:721-723. 1910; also The botanical evidence of coastal subsidence. *Science N.S.* 33:300-302. 1911.

investigate botanical phases of the question, and my modest excursions into the realms of a sister science have been made most profitable because of the courtesy and generous assistance of some of my botanical colleagues. I shall be glad if the observations here set down prove of interest to some of the many students of botany who have noted the indications of changes of level afforded by plant life along the coast.

Throughout this article the expression "recent subsidence" is employed to designate subsidence within the last few thousand years, and "remote subsidence" to designate a sinking of the land which occurred more than 4000 or 5000 years ago. One may divide the botanical evidences of recent subsidence of our Atlantic coast into three classes, according as they are (1) wholly fictitious appearance of changes of level; (2) phenomena produced by local changes in tidal heights without any real change in the general level of either land or sea; and (3) phenomena really produced by a sinking of the land, but produced so long ago that they cannot properly be cited as proofs of a subsidence within the last few thousand years. Let us first consider those supposed proofs of recent subsidence which are based on

### 1. Fictitious appearance of changes of level

#### STANDING FORESTS KILLED BY THE INVASION OF THE SEA

At many points on the Atlantic coast one may observe large numbers of trees killed by salt water so recently that they still stand erect, and even retain their branches. These trees have often been cited as a convincing proof of the recent progressive subsidence of the land. Among the localities where standing forests killed by the sea have been supposed to show with special clearness a recent subsidence of the land, I will mention but a few. GANONG<sup>5</sup> in one of his series of "Notes on the natural history and physiography of New Brunswick," speaking of the low shores of South River, near Pokemouche, says, "in places the dead forest trees still standing with their roots immersed by the highest tides,

<sup>5</sup> GANONG, W. F., On the physiographic characteristics of the Pokemouche and St. Simon rivers. Bull. Nat. Hist. Soc. New Brunswick 5:524-526. 1906.

afford striking evidence of the rapid subsidence this coast is undergoing." ABRAHAM GESNER<sup>6</sup> described standing forests of beech, birch, and maple, killed by the seawater which overflowed their roots, as a proof that the coasts of Cascumpeque Harbor in Prince Edward Island had undergone the most recent subsidence of which he had knowledge. COOK, in the paper referred to in the first paragraph of this article, describes dead forests still standing, found on several parts of the New Jersey coast. Sir CHARLES LYELL<sup>7</sup> saw in standing forest trees killed by the tide near the mouth of Cooper River, South Carolina, a proof of very modern subsidence of that coast.

The region described by GANONG has been examined by GOLDTHWAIT, with whom I have had the privilege of cooperating in the work along the coast of southeastern Canada. After a careful study of the dead trees on this part of the coast, GOLDTHWAIT<sup>8</sup> has reached the conclusion that death has resulted in some cases from fire, and in others from a local rise in the high tide level after the manner indicated in section 2 below. I have myself made a careful study of the Cascumpeque Harbor locality and find that the dead trees described by GESNER may be reasonably explained without imagining a subsidence of the coast.<sup>9</sup> Three distinct causes have operated to kill the forest of this portion of the shore. On the outer side of the barrier beach the waves are cutting away the shore and hurling the sands up into the forest. During storms the waves break over the sandy accumulation, and the salt water saturates the sand about the roots of the trees and is ponded back in the low depressions, remaining long enough to kill the trees. Forests killed in a similar manner are found on parts of the North Carolina capes. Inside Cascumpeque Harbor the small waves of the bay have gently sapped the mainland shores, removing the

<sup>6</sup> GESNER, ABRAHAM, On elevations and depressions of the earth in North America. *Quar. Jour. Geol. Soc. London* 17:381-388. 1861.

<sup>7</sup> LYELL, CHARLES, *Travels in North America*. London. 1845. 1:174-175.

<sup>8</sup> GOLDTHWAIT, J. W., Supposed evidences of subsidence of the coast of New Brunswick within modern times. Unpublished manuscript to appear in an early issue of the *Victoria Museum Bulletin*.

<sup>9</sup> JOHNSON, D. W., The shore line of Cascumpeque Harbor, Prince Edward Island. *Geog. Jour. London* 42:152-164. 1913.



earth from about the roots of the trees along the low coast and thus exposing them to salt water. The barrier beach which separates the bay from the ocean is interrupted by several tidal inlets; and a variation in the number and width of these inlets has permitted a local rise in the high tide surface with a consequent invasion of the forest by the salt water (see section 2 below). The dead forests along the coasts of New Jersey, the Carolinas, and Georgia, many of which I have examined, are most frequently to be explained

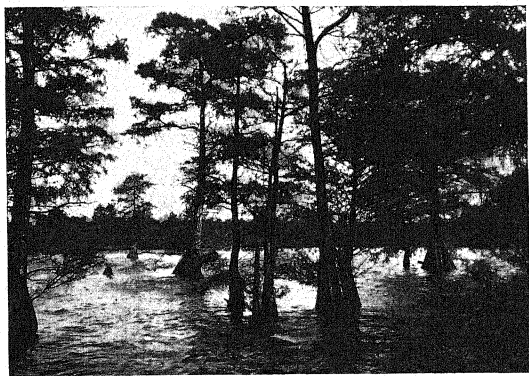


FIG. 1.—Live trees in Albemarle Sound, giving fictitious appearance of coastal subsidence.

as the result of such local fluctuations of high tide level. I have seen no case where the killing of the trees could safely be ascribed to a sinking of the coast. On the contrary, the localization of the dead trees at those points on the coast most favorable to the operation of the local causes mentioned above, and certain others described later, is sufficient evidence that their death is not the result of a general subsidence of the land.

As a variant of the above type of evidence, one may class the occurrence of live trees standing in deep water at the heads of

sounds and bays, where the water is too fresh to readily kill trees which have reached maturity. It is evident that these trees could not have commenced to grow in water 5 or 10 feet deep, and it was therefore with especial interest that I learned from Dr. C. A. DAVIS that great numbers of such trees occurred near the headward portion of Albemarle Sound, especially in the vicinity of Elizabeth City, North Carolina, affording, in his opinion, exceptionally good proof of recent coastal subsidence. On visiting the locality I found hundreds of live cypresses standing in water which was often over 5 feet in depth; but the spreading base of these trees was just above water level at the same elevation as on the adjacent low shore (fig. 1); while the underwater parts divided into spreading roots between which an oar could be readily passed. It was quite evident that the trees had grown, like their neighbors, on a low coast composed of peaty soil; and that the washing away of the soil by waves had left the trees standing out in the water. This interpretation was confirmed by the finding of occasional islets of peat about some of the isolated trees. There was no indication of any change in the relative level of land and sea.

#### SUBMERGED STUMPS

Closely allied to the foregoing botanical evidence of subsidence is that furnished by submerged stumps. These are found along all parts of the Atlantic coast, at depths varying from a few inches below high tide to ten feet or more below low tide level, and have been repeatedly cited by both botanists and geologists as conclusive proofs of recent subsidence. It is hardly necessary to cite specific descriptions of these submerged stumps, as they are such common features along our shores.

A study of the submerged stumps convinces one that there is a great variety of ways in which they may be produced independently of coastal subsidence. Along the low shores of South Carolina and Georgia the small waves formed in the passage between the "Sea Islands" continually undermine trees and let them down into the salt water. So gently does the process operate that the trees often remain erect; and I have seen all stages from trees still living on the edge of the shore 0.5-1 meter above tide,

through others whose broad spreading roots were half undermined, thus allowing the trees to incline forward and sink toward the lower level, to still others which had been wholly undermined and had tipped back to a nearly vertical position, standing erect but dead in the salt water. These trees later break off at the water line and give upright submerged stumps. The fact that dead trees and submerged stumps are often produced in this manner was clearly recognized by TUOMEY<sup>10</sup> in his interesting report on the geology of South Carolina. This same author<sup>11</sup> likewise emphasized the fact that many so-called submerged stumps are merely tap roots of certain trees which descend to a great depth. The loblolly pine has a tap root as large as its trunk which runs down 2 or 3 meters, and then sends off smaller roots. A forest of such trees growing on a low coast may be attacked by the waves, and as the earth is removed the trees die and finally break off at or below water level. In this way deeply submerged "stumps" are produced which will seem to the ordinary observer a convincing proof of subsidence.<sup>12</sup> Fig. 2 is a diagrammatic representation of several stages in this process, which we found particularly well shown in the "Sea Islands" of Georgia.

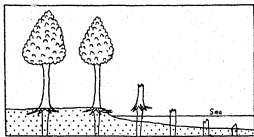


FIG. 2.—Submerged stumps resulting from normal retrogression of the shore line.

Where the sea is cutting into the "Black Bank," a peat bog near Cascumpeque Harbor, many stumps had been washed out of the bog, transported by waves and currents some little distance, and left stranded, often in an upright position, on the beach and in the shallow water of the bay. Submerged stumps, due to a local rise of the high tide level, to the compression of peat bogs caused by a lowering of the ground-water level as the waves cut into the shoreward side of such bogs (fig. 3), to the compression of peat

<sup>10</sup> TUOMEY, M., Report on the geology of South Carolina. Columbia. 1848. p. 194.

<sup>11</sup> *Op. cit.*, 195.

<sup>12</sup> LYELL, CHARLES, A second visit to the U.S. of North America. 2d ed. London. 1850. 1:316-317.

deposits under the weight of barrier beaches (fig. 4), and to other causes, have been observed at many points along the coast. The more one sees of this type of evidence the more does he realize its unreliability.

#### SUBMERGED PEAT

Another botanical evidence of subsidence, frequently appealed to with confidence by those who believe in recent subsidence of the Atlantic coast, is the submerged peat exposed at many points along the shores, sometimes a little below high tide, often a con-

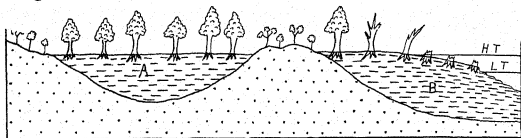


FIG. 3.—Submerged peat and stumps produced by an invasion of peat bogs by the sea; HT, high tide; LT, low tide.

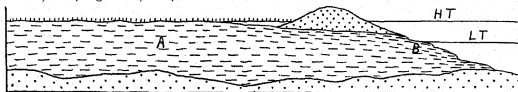


FIG. 4.—Submerged peat outcropping at low tide (B) compressed by weight of barrier beach, which is encroaching on salt marsh peat deposit (A); HT, high tide; LT, low tide.

siderable distance below low tide. Such deposits may consist of the remains of fresh water vegetation, or of the remains of marine plants; and both types of deposits have been cited as proofs of a recent sinking of the coast. The fresh peat is frequently overlaid by salt peat; sometimes the reverse is the case; while in other places one or the other type of peat occurs alone. It is uncommon, however, to find such a repeated interstratification of fresh peat with marine deposits as SKERTCHLEY<sup>13</sup> has described for the English fenland, or CAYEUX<sup>14</sup> for a portion of the French coast, although

<sup>13</sup> SKERTCHLEY, S. B. J., *The geology of the fenland*. Memoir Geol. Surv. Eng-land and Wales. London. 1877. pp. 145-151, 172-174.

<sup>14</sup> CAYEUX, L., *Les tourbes immergées de la côte Bretonne dans la région de Plougasno-Primel (Finistère)*. Bull. Soc. Géol. France IV. 6: 142-147. 1906.

essentially the same conditions developed on a small scale are occasionally encountered on our coast.

Evidently the fresh peat cannot have formed in its present position, exposed to marine action. Indeed, it has been argued that such peat containing upright stumps cannot form in depressions below high tide level back from the shore line because the level of the ground-water would cause such depressions to contain ponds of water in which trees would not grow. Hence it is concluded that submerged fresh peat is a proof of recent coastal subsidence. This conclusion seems to me open to criticism on several grounds. In the first place, "floating bogs" formed of sphagnum and carrying trees of considerable size upon them sometimes cover the surfaces of ponds. The sinking of such a bog, as the trees increase in size or as new material is added to its upper surface, would carry upright stumps down below sea-level. Decomposition of bogs to produce such a semi-liquid mass as is often found under their surfaces might permit stumps to sink slowly to the bottom and remain upright there. The possibility of some such history for a peat bog encroached upon by the sea must be definitely excluded by those who would employ these bogs as a proof of coastal subsidence. In the second place, it should be noted that the lower portions of such bogs may be of very great antiquity; even if they formed above sea-level and were carried downward by subsidence of the coast, this event may have taken place many thousands of years ago. Hence such submerged bogs should not be offered as a proof of recent subsidence (within the last 2000 or 3000 years), as has so often been done. In the third place, when such a bog is encroached upon by the sea, the level of the ground-water table in the bog, formerly at or near its surface, is rapidly lowered. Near the seaward margin of the bog the ground-water table may decline to mean sea-level; and, right at the margin, to low tide level when the tide is out. As a result of this removal of water, the surface of the bog is rapidly lowered, carrying down with it trees which are killed by exposure to high tide (fig. 3). How extensive such a settling of the surface may be, is suggested by fig. 5, which represents the result of an artificial lowering of the ground-water level. Furthermore, the alternate

submergence and draining of the bog removes so much of its content that the surface may even slope down to a level considerably below that of low tide. Submerged deposits of fresh peat containing upright stumps, therefore, are not to be regarded as a conclusive proof of subsidence, either remote or recent.

The salt marshes of a portion of our coast are underlaid by peat, often remarkably pure and reaching a depth of 6 meters or more, composed largely of the roots of *Spartina patens* and other

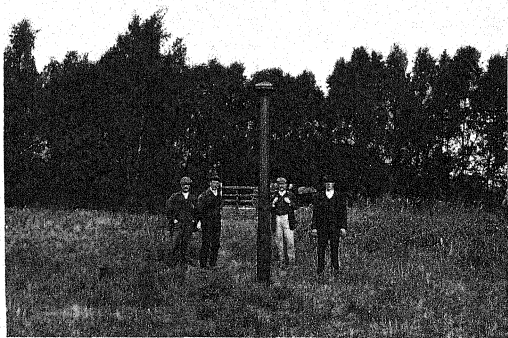


FIG. 5.—Settling of peaty land due to artificial lowering of ground-water level in the fenland of England; in 1848 the surface of the land was even with the top of the post.

salt marsh grasses which grow only near the high tide level. In 1862 MUDGE<sup>15</sup> called attention to this structure of the salt marshes along the New England coast, and showed that the deeply buried portions of the peat must have formed at high tide level. Their present position he attributed to an undermining of the subsoil by fresh water, known by borings to descend from the upland and to pass through the sand found below a clay bed under the marsh

<sup>15</sup> MUDGE, B. F., The salt marsh formations of Lynn. Essex Inst. Proc. 2:117-119, 1862.

near Boston; an explanation which seems hardly competent to account for such a widespread phenomenon. More recently DAVIS<sup>16</sup> of the United States Bureau of Mines, an expert in the examination of peat deposits, has placed especial emphasis upon that feature of salt marshes described by MUDGE, and believes that he has found therein a conclusive proof of a gradual subsidence of the Atlantic coast, probably not faster than a foot a century, continuing up to the present time. Others have likewise thought that they found in submerged salt peat a proof of recent subsidence, and have cited the outcropping of such peat at low tide on the seaward side of barrier beaches, bearing the impressions of the hoofs of cattle and horses and of wagon wheels, as certain proofs of very recent marked subsidence.

It is well known that the attack of the waves often drives a barrier beach inward over the salt marsh. The enormous weight of the beach necessarily results in a compression of the peat deposit, so that the surface of the latter is exposed near or below low tide level on the seaward side of the beach (fig. 4). On the coast near Boston a barrier beach has been driven back over a salt marsh more than 70 meters in twelve years. Today the former surface of the meadow, with the wheel tracks of an old road, impressions of horses hoofs, and the stumps of trees which had gained a foothold on the marsh inside of the beach, are all exposed at low tide on the seaward side of the beach. Those who would interpret this as a result of coastal subsidence must admit a subsidence of perhaps two meters in twelve years, of which there is no corroborating evidence whatever. In fact, the bending down of the former surface of the marsh is readily apparent where the peat outcrops toward the sea; and the fact of extensive compression is shown by two sections taken through the peat deposit; one in the marsh a short distance back of the beach, revealing about four meters of relatively soft peat; the other near low tide level, showing but one meter of very dense, compact, tough peat. The seaward slope of the former marsh surface may be obscured back of the beach by more recent deposits built up to high tide level (fig. 4).

<sup>16</sup> BASTIN, E. S., and DAVIS, C. A., Peat deposits of Maine. Bull. 376, U.S. Geol. Surv. 19-21. 1909; also DAVIS, C. A., Salt marsh formation near Boston, and its geological significance. *Economic Geology* 5:625. 1910.

In sections 2 and 3 below we will inquire further into the reliability of salt peat below sea-level as a proof of recent subsidence.

## 2. Phenomena produced by a local rise in the high tide level

It has seemed necessary to treat the fictitious appearances of changes of level as fully as has been done above, because of the widespread belief in the value of such phenomena as proofs of coastal subsidence. The local fluctuations of high tide level, now

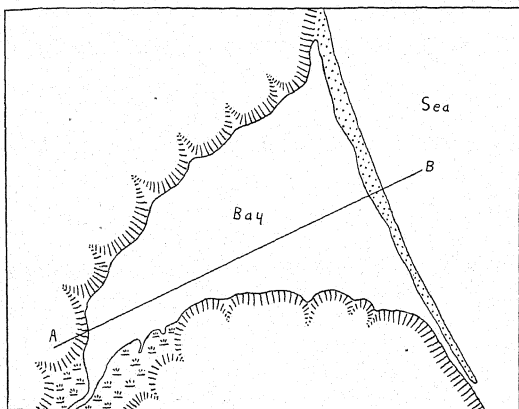


FIG. 6.—Bay separated from the open sea by a barrier beach

to be discussed, are of much greater importance, but may be explained in a shorter space. The principle of these fluctuations will be readily apparent from figs. 6-8.

On a tidal coast, if we have a bay like that shown in fig. 6, almost separated from the open sea by a barrier beach, but connected with it by a narrow tidal inlet, the waters of the rising tide in the sea will pass through the tidal inlet with so much difficulty that the surface of the bay will rise much more slowly than the surface of the sea. When the tide in the sea has reached its



maximum, and has begun to fall, the surface of the bay will still remain much lower. When it is low tide in the sea, the water in the bay will remain at a higher level, because this water cannot escape fast enough to maintain equality of levels between the two water bodies. Hence, high tide level in the bay is lower than high tide level in the sea. This is shown in fig. 7, which represents a cross-section of such a bay as fig. 6 in the direction *AB*. It is evident that around the shores of the bay, trees and other fresh water vegetation will grow down to the level of the high tide of the bay, and thus *below* the high tide level of the adjacent sea. Salt marshes in the bays will likewise grow up to the high tide level of the bay, farmers will build dikes to reclaim their marshes

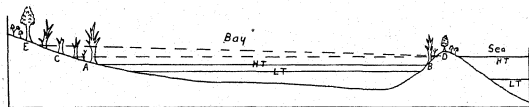


FIG. 7.—Diagram showing fictitious subsidence of the coast; as long as the barrier beach (*D*) nearly closes the mouth of the bay, high tide (*HT*) in the bay is lower than high tide in the open sea; trees grow down to this lower level (*AB*) along the shores of the bay; when the barrier beach is broken through or removed, high tide in the bay rises as high (*CD*) as the open sea, and all the trees between the levels *AB* and *CD* are killed by the salt water; if the bay narrows going inland, the tide is forced to rise even above the level it attains in the open sea, or to the position *ED*, and at the head of the bay all the trees between *A* and *E* are killed; in addition to these submerged forests, other fictitious indications of subsidence are thus produced.

at this same level, and in other ways the level will be so marked as to render readily perceptible any increase in the height of the tides.

Now let us consider the consequences which must follow if storm waves make a large breach in the barrier beach. With free access to the bay through the larger opening, the tidal waters will at once rise as high in the bay as in the open sea (*CD*, fig. 7). All trees whose bases are below the line *CD* will be washed by the tides and killed. The standing forests of dead trees will later be represented by submerged stumps. Dikes raised by the farmers will be overflowed by the tides. The surface of the salt marsh will build up to the new high tide level, enveloping both stumps

and dikes. Fresh water peat, formerly beyond the reach of salt water, may now be buried under a layer of salt peat. In short, most of the phenomena usually cited as proofs of general coastal subsidence will be produced by a local rise of the high tide caused by change in the form of the shore line. If the bay narrows inland as shown in fig. 6, the tidal wave will increase in height as it advances, so that the level of high tide at the head of the bay will rise far above that of the open sea (*ED*, fig. 7). In this case all the trees between *A* and *E* will be killed at the head of the bay, and the appearance of subsidence will be unusually pronounced.

Fig. 8 represents the consequences of the opposite type of change. When the bay was open to the sea, the waves cut a cliff (*C*) and bench (*B*). But the construction of a barrier beach (*D*) has so

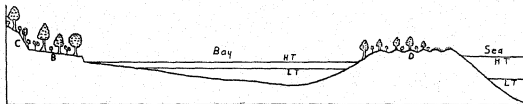


FIG. 8.—Diagram showing fictitious elevation of the coast; before the barrier beach (*D*) was constructed, the tide in the bay rose as high as in the open sea, and the cliff (*C*) and bench (*B*) were carved by the waves; since the building of the barrier beach, high tide (*HT*) in the bay is lower than in the ocean, the cliff and bench are no longer reached by the waves, and appear to represent an "elevated shore line"; the uniform altitude of the beach ridges on the barrier beach shows that the relative level of land and sea have long remained constant.

reduced the level of high tide in the bay that the waves no longer reach the cliff or the inner part of the bench. These become covered with trees and other fresh vegetation, and constitute what is usually called an "elevated shore line." Cliffs and benches of this origin have been cited as proofs of recent coastal elevation.

In applying the above principle to the interpretation of supposed elevations and subsidence of the land, the following points should be kept in mind:

a) If, instead of a sudden rupture of the barrier beach, we have a gradual enlargement of the inlet, the shores of the bay will appear to undergo slow and progressive subsidence. The gradual closing of an inlet, or the progressive shifting of its position, will likewise

cause the appearance of slow changes of level. Such changes are more common, but less striking, than those which are more sudden.

b) The total apparent subsidence produced in this manner may considerably exceed one-half of the tidal range in the adjacent sea. A considerable thickness of high tide salt peat may thus be produced without coastal subsidence.

c) Half-tide level does not necessarily remain the same after the change in high tide level.

d) The application of the principle here set forth is not restricted to bays of the type shown in fig. 6. Vast lagoons parallel to the coast, such as those of Long Island and New Jersey; open bays whose mouths are being widened by wave erosion; salt marshes traversed by meandering tidal channels; the intricate network of passes between the low and changeable islands of South Carolina and Georgia, or parts of the Holland coast; all these present favorable conditions for local changes of tide level consequent upon changes in the width, length, or depth of the tidal channels.

e) Many bays now open to the sea were once doubtless more or less nearly closed by barrier beaches. This is especially true along glaciated shores where the waves and currents have effected in post-glacial time, and are still effecting, comparatively rapid changes in the form of the shore line.

f) Appearances of subsidence predominate over those of elevation because marsh deposits tend to sink to the new level when high tide level is lowered; because the immediate destruction of fresh water vegetation by salt water when the high tide limit is raised is more striking than the slow recovery of marine area by fresh vegetation when the high tide level is lowered; and because in the cycle of shore line development retrograding exceeds prograding, and retrograding tends to carry higher tide levels into low lands where apparent changes of level are most easily recognized.

In order to determine how far conditions along the Atlantic coast were favorable to such future changes in high tide level as would produce apparent coastal subsidence, we made careful comparison of the height of the same high tide in partially closed bays, in lagoons, and in tidal creeks of salt marshes on the one hand, and in the ocean on the other hand, at a large number of

points along the coast from New Hampshire to Florida. The results of these surveys show that such favorable conditions occur abundantly along the entire coast. Differences of level in the high tide of the two contrasted water bodies amounting to nearly a meter were found, and from other surveys farther north it is known that still greater differences occur.

That such local changes of high tide level have occurred in the past is equally evident. At Scituate near Boston, the storm of 1898 made a large opening in a barrier beach which formerly nearly



FIG. 9.—Trees killed by local rise of high tide, near Scituate, Mass., giving fictitious appearance of coastal subsidence.

closed the mouth of a small bay. The high tide level immediately rose, according to the inhabitants, more than half a meter, and extensive areas of growing trees were invaded by salt water, the trees now standing erect but dead (fig. 9). Dikes built to reclaim portions of the former marsh surface are overflowed by the tides, and the marsh is building up to the new level.

In 1811 a break in a barrier beach a short distance to the south is said to have resulted in the death of many trees, the stumps of which have recently been extracted from the shallow shoreward portion of the marsh. At Cascumpeque Harbor, Prince Edward

Island, there have been several recent changes in the number and position of the tidal inlets connecting with the sea, and the inhabitants date the death of certain of their trees to a new inlet opened some years ago. Along the New Jersey coast the general surface of the marsh slopes downward toward the land, and on the Delaware Bay section of this shore the waves are cutting rapidly into the marsh, which is unprotected by barrier beaches. As a result of the consequent shortening of the meandering tidal creeks, the tides rise progressively higher and higher toward the heads of the creeks; the salt marsh builds gradually up to the new level of high tide, encroaching on the upland, killing the trees, and producing other evidence of progressive subsidence of the land. In the sea islands of South Carolina and Georgia we observed a number of more or less restricted localities where forests had been killed by a rise of high tide level following changes in size and position of tidal channels and bars, and one place where the death of the trees is dated from the cutting of a canal between two tidal channels. On the other hand, appearances of elevation of the land caused by a local lowering of the high tide level are not lacking. "Elevated" cliffs and benches of this origin were observed at a number of points on the coasts of Massachusetts, New Jersey, North Carolina, and Florida.

### 3. Phenomena due to remote subsidence

Frequent warnings have been uttered, most ably by SUESS, against the danger of confusing evidences of ancient changes of level with evidences of recent changes of level. Yet this error is found all too often in writings on this subject even today. Stumps deeply buried in the salt marshes are correlated with the invasion of cultivated fields by the tides; deeply buried salt peat is correlated with the dying of forests along the shore today; and on the basis of such correlations it is argued that we are in the presence of a great movement of subsidence which has continued uninterruptedly throughout recent time. It has even been argued that the embayed or drowned river valleys of the Atlantic coast are a conclusive proof of recent subsidence.

It is of the highest importance to recognize the possibility that deeply submerged stumps and peat, embayed valleys, and similar

evidence may have been produced by a downward movement of the land which entirely ceased thousands of years ago; and that they may be wholly unrelated to those evidences which may properly be designated as recent. Attacking the problem from this point of view, I have been unable to find a single evidence of recent change of level on the Atlantic coast which may not be reasonably explained either as a fictitious appearance of changes of level, or as the result of a local fluctuation in the level of high tide. On the other hand, I have been unable to find a single conclusive proof of a change of level which is not in all probability of considerable antiquity.

The best example of deeply submerged stumps which I have seen on the entire coast is that so well described by DAWSON<sup>17</sup> at the head of the Bay of Fundy. The position of these stumps indicates a veritable subsidence of the land, but they have been buried under the great thickness of silt since the embayment of the region, and have been brought to light again in recent time by a shifting of a tidal channel. The position of the more deeply buried portions of the salt peat under the salt marshes is most reasonably explained as the result of coastal subsidence; but this peat may well be of considerable antiquity and probably dates well back toward the early part of post-glacial time at least.

In closing this account of the relation of botanical phenomena to the problem of recent coastal subsidence, I desire to call attention to the application of some of the above considered principles to certain evidence lately presented by BARTLETT.<sup>18</sup> According to this author, a peat bog at Quamquisset Harbor, near Woods Hole, occupies a kettle hole and represents successive layers of vegetation continuously built up to the surface of a ground-water table which rose higher and higher as the land subsided. This subsidence required something over 2000 years, and is still in progress, the sea having recently cut into the bog deposit.

Irrespective of the question as to whether the Woods Hole

<sup>17</sup> DAWSON, J. W., On a modern submerged forest at Fort Lawrence, Nova Scotia. Quar. Jour. Geol. Soc. London 11:119-122. 1855.

<sup>18</sup> BARTLETT, H. H., The submarine *Chamaecyparis* bog at Woods Hole, Massachusetts. Rhodora 11:221-235. 1909; also Botanical evidence of coastal subsidence. Science N.S. 33:29-31. 1911.

bog affords "incontrovertible evidence" of recent subsidence, as BARTLETT believes, that author is to be congratulated on having set forth in a clear manner the series of changes which will occur in a bog occupying a depression closed from the sea, on a coast which is really subsiding. But the particular case to which BARTLETT applies this principle seems to me unfortunate. In the first place, one must question whether the depression in which the bog deposit occurs is really a kettle hole. There are, to be sure, many kettle holes in the terminal moraine of this region; but the Quamquiset Harbor bog near Woods Hole appears to occupy a normal stream channel in drift which is probably older than the moraine, the channel having been somewhat modified by later ice action. Like many other similar channels which I have studied, this one was probably open to the sea, in which case the entire history of the bog must have been quite different from that imagined by BARTLETT.

Even if the depression were a kettle hole, the validity of BARTLETT's argument must still depend upon three further assumptions, all of which seem to me open to question: (1) the *Chamaecyparis* stumps occur in place from the bottom to the top of the deposit; (2) coastal subsidence is the only theory competent to explain such a succession of stumps in place; (3) the lower as well as the uppermost layers of the deposit are of recent date (i.e., formed within the last 2000 or 3000 years). Stumps certainly occur in place near the surface of the bog, and extensive soundings verified the abundance of wood found by BARTLETT in depth. But all of the cores which I was able to bring up by numerous tests showed the grain of the wood transverse to the core, indicating that I had encountered only trunks, branches, or roots lying horizontally. Of course, the chances of encountering the end of an upright stump are not great, but the fact that a day's almost continuous sounding failed to discover an undoubted stump in depth shows how difficult it must be to prove that the bog consists largely of stumps *in situ*. BARTLETT presents no evidence of the existence of such stumps in depth, aside from the fact that he encountered buried wood. In a number of cases I determined the form of the buried wood by abundant closely spaced soundings, and invariably found greatly elongated pieces, evidently logs or branches.

Even if stumps occur in place throughout the deposit, they cannot be cited as incontrovertible evidence of subsidence until we make sure that nature can in no other manner produce such a succession of stumps in place. It has occurred to me that floating bogs bearing trees might sink as new accumulations of material in place would be carried downward to the bottoms of ponds or lakes. Several botanical friends to whom I have appealed tell me that buried stumps might well be produced in this manner. PENHALLOW<sup>19</sup> describes a bog which, according to his interpretation, has had such a history. Surely then we are justified in doubting the assumption that stumps in place deep down in a kettle hole bog are conclusive proof of a change in the relative level of land and sea.

But if we granted that the phenomena cited by BARTLETT could be accepted as a proof of coastal subsidence, we must still ask for some satisfactory evidence of the age of the lower part of the deposit before we can accept it as a proof of recent subsidence. The upper part of the deposit may well be of recent date, and yet not be the result of coastal subsidence. If any part of the deposit proves subsidence it is the lower part, which cannot have been affected by changes in tidal levels. But are we sure that this lower part is of recent date? Surely those familiar with the antiquity of some of the peat bogs of Europe, in which wood and other substances are still well preserved, will hesitate to affirm that the lower part of a given peat bog must be of recent date as here defined.

When critically examined, neither the botanical nor other evidence of recent coastal subsidence seems to me conclusive. On the other hand, the physiographic evidence, so far as I have been able to analyze it, indicates a long period of coastal stability. The evidence in favor of stability I have already briefly outlined elsewhere, and I will present a more detailed account of it in a forthcoming report on the Shaler Memorial investigation of shore line changes along the Atlantic coast.

COLUMBIA UNIVERSITY  
NEW YORK CITY

<sup>19</sup> PENHALLOW, D. P., A contribution to our knowledge of the origin and development of certain marsh lands on the coast of New England. Roy. Soc. Canada, Proc. and Trans. III. 1:33-34. 1907.



## WESTERN PLANT STUDIES. II

AVEN NELSON AND J. FRANCIS MACBRIDE

PENTAMERIS.—As shown by PIPER in his Flora of Washington (Contrib. Nat. Herb. 11:122), the name *Danthonia* is not available for the American species that have passed under that name. In choosing among the several later names that have been proposed, he selects *Merathrepta* Raf. in Seringe, Bull. Bot. 1:221. 1830, apparently because the type species of the genus was *M. spicata*, an American species closely congeneric with ours. But will this fact permit our ignoring *Pentameris* Beauv. Agrost. 92. t. 18 1812, the type species of which is accepted as a *Danthonia*, as that genus has until lately been understood? Recent students of this genus recognize two sections, but so long as both remain as sections merely, all the species must be retained under the oldest available name that has had generic rank. This seems to be *Pentameris*, published as shown above, and attested by the many species subsequently referred to it. The types of the two sections are given along with the American species, and one other to show the synonymy.

PENTAMERIS THUARII Beauv. Agrost. 93. t. 18. 1812.—*P. tortuosa* Nees, in Linnaea 7:311. 1832; *Danthonia tortuosa* Trin. Sp. Gram. t. 68. 1828–1836; not *D. Thuarii* Desv. Opusc. 99. 1831.

*Pentameris provincialis*, n. comb.—*Danthonia provincialis* DC. Fl. Fr. ed. 3. 3:33. 1809 (?).

*Pentameris americana*, n. comb.—*Danthonia americana* Scribn. U.S. Dept. Agric. Circ. Agrost. 30:5. 1901; *D. grandiflora* Philippi, Anal. Univ. Chile 568, 1873, not Hochst. 1851.

*Pentameris californica*, n. comb.—*Danthonia californica* Boland. Proc. Cal. Acad. 2:182. 1863.

*Pentameris compressa*, n. comb.—*Danthonia compressa* Aust. Bull. Torr. Bot. Club 3:21. 1872.

*Pentameris epilis*, n. comb.—*Danthonia epilis* Scribn. U.S. Dept. Agric. Circ. Agrost. 30:7. 1901.

*Pentameris grandiflora*, n. comb.—*Danthonia grandiflora* Hochst. ex A. Rich. Tent. Fl. Abyss. 2:418. 1851.

*Pentameris intermedia*, n. comb.—*Danthonia intermedia* Vasey, Bull. Torr. Bot. Club 10:52. 1883.

*Pentameris sericea*, n. comb.—*Danthonia sericea* Nutt. Gen. Am. 1:71. 1818.

*Pentameris spicata*, n. comb.—*Avena spicata* L. Sp. Pl. 80. 1753.

*Pentameris thermale*, n. comb.—*Danthonia thermale* Scribn. U.S. Dept. Agric. Circ. Agrost. 30:5. 1901; *Merathrepta pinetorum* Piper. Contrib. Nat. Herb. 11:122. 1906.

*Pentameris unispicata*, n. comb.—*Danthonia unispicata* Thurb. Bot. Cal. 2:294. 1880.

*Allium textile*, n. n.—*A. reticulatum* Fraser in Mem. Wern. Soc. 6:36. 1827; not *A. reticulatum* J. and C. Presl. Fl. Cech. 73. 1819.

Our collections made in 1912 show that this species has a wider distribution than heretofore assigned to it. Specimens having been secured on the Snake River, at Shoshone Falls, it seems probable that it may extend quite into eastern Oregon.

*ALLIUM FIBRILLUM* Jones, Contrib. Bot. 10:24. 1902.—*A. collinum* Dougl. in Wats. Proc. Am. Acad. 14:228. 1889; not *A. collinum* Guss. in Tenore Syl. Pl. Vas. Fl. Neap. 169. 1831.

PIPER, in his Flora of Washington, is undoubtedly right in citing the specimens of HORNER (nos. 190, 193, and 470) from the Blue Mountains (type locality) as representative of the heretofore poorly understood species published by WATSON for DOUGLAS.

*Allium incisum*, n. sp.—Bulbs large, 1-1.5 cm. broad, outer coats brown or pinkish, reticulation obscure: scapes 5-8 cm. high, stout, 1.5-2.5 mm. broad, narrowly winged: leaves 3-5 mm. broad, thick, and slightly falcate, attenuate to a long point, distinctly exceeding the scape, rather strongly nerved: spathe 2-several valved or at least one or all of the bracts deeply incised so as to appear distinct: bracts and their divisions ovate or broadly lanceolate at base, gradually long-acuminate, the margins of each often incurved and united near the tip: umbel globose, many-flowered: pedicels slender and flexuous, 1-2 cm. long: flowers white: segments shorter than the stamens, narrowly lanceolate,

acute, 1-nerved, not rigid but weak and crinkled in fruit: capsule very obscurely crested.

This species seems unique in its incised bracts. No. 1790 from barren gumbo clays, probably soft in the spring but becoming very hard, wholly devoid of other vegetation, House Creek, Owyhee Co., Idaho, June 29, 1912, is the type.

**Calochortus maculosus**, n. sp.—Bulb narrowly ovate, about 1 dm. below the surface, this and the underground part of the stem covered with coarse thick barklike coats: stems rather stout, smooth, 3-4 dm. high: leaves several (4-6), all abruptly expanded and scarious at the sheathing base: flowers 2, one on a spreading pedicel opposite the last bractlike leaf and also bracted; the other surmounting the main stem on a longer pedicel: sepals narrowly lanceolate, gradually long acuminate, 4-5 cm. long, greenish within and without: petals longer, 4.5-6 cm. long, abruptly acute and slightly crenate, white, or very pale blue, with a green band from apex to the large purple splotch above the yellow cuneate base, on which is the large gland, all the yellow area with long crinkled hairs: anthers 2 or 3-ribbed, obtuse, yellow, about equaling the filaments: capsule narrowly oblong.

This, the fourth member of the *macrocarpus* group, is easily distinguished from *C. bruneae* Nels. and Macbr., its nearest relative, by the hairy lower part of the petal and the color, and from the others by the large purple blotch. In aspect it somewhat resembles the *nitidus* group, from which the elongated capsule readily separates it. The characteristic green band and the few-ribbed anthers are some of the characters which forbid its being referred to the *Nuttallii* group. The type is no. 2727 by HENDERSON, in loose soil on the hills near Lewiston, Nez Perce Co., Idaho, June 17, 1894.

EPIPACTIS Adans. evidently cannot stand (see TORRE and HARMS Gen. Siph. nos. 1482 and 1504, and DRUCE's illuminating paper, Bull. Torr. Bot. Club 36:543. 1909). But is *Helleborine* more truly available? This question it seems to us DRUCE has partly answered by his own arguments and statements of fact. We note that while *Hel'eborine* was in use before 1753, it was not published *with species* until DRUCE gives us the list that falls into the genus, restricted as now understood. DRUCE's aim was to bring his work into harmony with the Vienna rules, but these deny valid ty to *uninominal* nomenclature, a principle reasserted by the Brussels Congress. This seems long to have been an unwritten law, for we

are told that JOHN HILL's work (British Herbal, 1756) remained unquoted even in the *Index Kewensis* because he had not adopted the *binomial* system. The only other name possibly available for the *Epipactis* of Adanson is *Limonias*, a name to be rejected primarily according to law, because it is a name published without species, and secondarily in harmony with common sense, because it might result in confusion, owing to *Limonia*, a valid genus in the *Rutaceae*, and *Limonium*, a genus in the *Plumbaginaceae*. There seems to be no alternative, therefore, except to select a new name, and in recognition of the splendid work by OAKES AMES in the *Orchidaceae*, we have chosen the following, to which all the species listed by DRUCE are transferred.

*Amesia*, n. n.—*Epipactis* Adans. Fam. 2:70. 1763, not *Epipactis* (Haller) Boehm. in Ludw. Definit. Gen. Pl. 1760, nor *Epipactis* Zinn, Cat. Pl. Hort. Acad. 85. 1757; *Helleborine* Hill and *Limonias* Ehrh. (see preceding paragraph).

*Amesia africana*, n. comb.—*Epipactis africana* Rendle, Jour. Bot. 33:252. 1895.

*Amesia atropurpurea*, n. comb.—*Epipactis atropurpurea* Raf. Car. 87. 1810.

*Amesia babianifolia*, n. comb.—*Epipactis babianifolia* Roxb. Hort. Bengal. 63. 1814.

*Amesia consimilis*, n. comb.—*Epipactis consimilis* Wallich, Cat. no. 7403.

*Amesia gigantea*, n. comb.—*Epipactis gigantea* Dougl. in Hook. Fl. Bor. Am. 2:202. 1830.

*Amesia latifolia*, n. comb.—*Epipactis latifolia* All. Fl. Pedem. 2:151; Sieb. in Sv. Vet. Akad. Nya Handl. 232. 1800.

*Amesia microphylla*, n. comb.—*Epipactis microphylla* Sieb. loc. cit.

*Amesia orbicularis*, n. comb.—*Epipactis orbicularis* C. Richt. Vehr. Zool.-Bot. Ges. Wien. 37:190. 1887.

*Amesia palustris*, n. comb.—*Helleborine palustris* Schrank, Fl. Monac. 2:190. 1814.

*Amesia papillosa*, n. comb.—*Epipactis papillosa* Franch. and Sav. Enum. Pl. Jap. 2:519. 1879.

*Amesia pycnostachys*, n. comb.—*Epipactis pycnostachys* C. Koch, Linnaea 22:289. 1849.

*Amesia somaliensis*, n. comb.—*Epipactis somaliensis* Rolf, Fl. Trop. Afr. 7:189. 1897.

*Amesia Thunbergii*, n. comb.—*Epipactis Thunbergii* Perry, Exp. Jap. 2:319. 1857.

*Amesia trinervia*, n. comb.—*Epipactis trinervia* Roxb. Fl. Ind. 3:455. 1832.

*Populus fortissima*, n. n.—*P. angustifolia* James ex Torr. Am. Lyc. N.Y. 2:249. 1828; not *P. angustifolia* Weinm. Elench. Hook. Palowsk. 451. 1824.

*Salix columbiae*, n. n.—*S. pyrifolia* Anders. Vet. Handl. Stockh. 6:162. 1867; not *S. pyrifolia* Schleich, Cat. Pl. Helv. ed. 3:26. 1815.

CARDIONEMA DC. Prod. 3:372. 1828.—*Pentacaena* Bartl. in Presl. Rel. Haenk. 2:5. 1830.

*Cardionema ramosissima*, n. comb.—*Loeflingia ramosissima* Weinm. Bot. Zeit 3:608. 1820; *Pentacaena ramosissima* Hook. and Arn. in Hook. Bot. Misc. 3:338. 1833; *Pentacaena polycnemoides* Bartl. in Presl. Rel. Haenk. 2:5. 1830.

*Cardionema camphorosmoides*, n. comb.—*Pentacaena camphorosmoides* Walp. Rep. 1:261. 1842.

*Cardionema rosetta*, n. comb.—*Pentacaena rosetta* Walp. loc. cit.

*Cardionema congesta*, n. comb.—*Pentacaena congesta* Benth. Pl. Hartw. 186. 1839.

*Cardionema andina*, n. comb.—*Pentacaena andina* Phil. in Anal. Mus. nac. Chile 26. 1891.

*Aconitum Howellii*, n. n.—*A. bulbiferum* Howell, Fl. N.W. Am. 25. 1897; not *A. bulbiferum* Reichb. Nebers. Acon. 55. 1819.

*Ranunculus reconditus*, n. n.—*R. triternatus* Gray, Proc. Am. Acad. 21:370. 1886; not *R. triternatus* Poir. Encyc. Supl. 4:662. 1815.

*Arabis crypta*, n. sp.—Biennial, stellate-pubescent throughout; stems one or two from the simple crown, branched above, 3 dm. high, greenish, the pubescence not so dense nor so perfectly stellate as that of the leaves and pods: basal leaves numerous, nearly linear, acutish, narrowed to a slender petiole, the midvein prominent, 2-3 cm. long; cauline distant, narrowly lanceolate, 3-nerved, slightly auriculate: flowers rather few, white or faintly tinted: petals 3-4

mm. long, scarcely twice the length of the green pubescent scarious-margined sepals: pods remote on the elongated rachis, white with the close pubescence, closely refracted at maturity, only 1-1.5 cm. long by 2 mm. broad or nearly that near the base, barely tapering to the apex: pedicels 2-3 mm. long: seeds few (2-6), orbicular, large, narrowly winged, uniserial or imperfectly biseriate.

Secured at Jarbridge, Elko County, Nevada, July 4, 1912; the habitat not noted.

*Idahoa*, n. n.—*Platyspermum* Hook. Fl. Bor. Am. 1:68. 1830; not *Platyspermum* Hoffmann, Genera Plantarum Umbelliferum 58. 1814.

*Idahoa scapigera*, n. comb.—*Platyspermum scapigerum* Hook. loc. cit.

This peculiar and well known crucifer occurs sparingly in Washington, Oregon, and Idaho, but apparently is met with most frequently in western Idaho (from the Panhandle to Nevada), hence the choice of name.

*Lepidium papilliferum*, n. comb.—*L. montanum papilliferum* Henderson, Bull. Torr. Bot. Club 27:342. 1900.—Biennial, densely papillose-pubescent, especially the stems; intricately branched, forming compact spherical clumps, consisting of a central stem with numerous lateral branches from its base to a point about one-third or one-half the height of the plant, where it in turn branches several times: lower leaves pinnatifid, even the uppermost deeply toothed: racemes short, dense: petals conspicuous, twice the length of the sepals: pods on slender, widely spreading, often even recurving pedicels, suborbicular, not at all narrowed to the notched apex, faintly glutinose-papillose: style well exerted.

No. 1068, Nampa, Idaho, distributed as *L. montanum* Nutt. is the type. No. 91, New Plymouth, and 880, Emmett, both by MACBRIDE and distributed as *L. Jonesii* Rydb., are typical.

*L. papilliferum* is distinguished at once from the species to which it has been referred by its distinctive habit of growth, its merely biennial duration and its unusual pubescence. *L. montanum* and *L. Jonesii* are perennial, the several stems spreading from a branched caudex, the plant possessing no main axis.

*Lepidium philonitrum*, n. sp.—Slender glabrate biennial with a slim tap root, 3-4 dm. high, the single sparingly branched stem simple below: leaves remote, the lower irregularly pinnate; the

cauline mostly trifid, the middle tooth the largest; the uppermost tridentate: flowers showy, the broad petals about twice the length of the sepals: fruiting pedicels slender, spreading, about three times the length of the pod: pod ovate, 3-4 mm. long by about 2 mm. broad, the narrow blunt apex slightly notched, the style less than twice the length of the scarcely involute apical wings.

No. 32, from alkaline bottom lands, Falk's Store, Idaho, May 17, 1910, by MACBRIDE, is the type. No. 2023 by CUSICK, near McDermitt, Oregon, may possibly be a pubescent form.

This is another member of the *alyssoides* group. Its pod allies it to *L. alyssoides* and *L. Jonesii*; its leaves to *L. papilliferum*; its aspect and habitat differ from all relatives.

Much has been made of differences shown by *Sisymbrium* and *Sophia* in the field; but, in common with many others, the writers are unable to see that each has vegetative characters so marked as to constitute generic differences. Believing that one genus should include them all, the following are transferred to *Sisymbrium*. In this connection it may be noted that *Descurainia* should replace *Sophia* only in the event that the latter is kept distinct.

*Sisymbrium paradisum*, n. comb.—*Sophia paradisa* Nels. and Ken. Proc. Biol. Soc. Wash. 19:155. 1906.

*Sisymbrium leptophyllum*, n. comb.—*Sophia leptophylla* Rydb. Bull. Torr. Bot. Club 29:239. 1902.

*Sisymbrium ochroleucum*, n. comb.—*Sophia ochroleuca* Woot. Bull. Torr. Bot. Club 25:455. 1898.

*Sisymbrium obtusum*, n. comb.—*Sophia obtusa* Greene, Leaflets 1:96. 1904.

COTYLEDON AND ITS SEGREGATES.—There being substantial agreement among botanists now in excluding the genus *Cotyledon* from this continent, the question arises as to the disposition of the species formerly referred to it and to *Echeveria*. This question has recently been answered by reestablishing *Echeveria* for a part and creating new genera for some of the more aberrant forms. It seems, however, that the differences relied upon to sustain some of these segregates are at best merely relative, and often inconsequential and unreliable, in this group. These characters are the shape of the leaves, the length or breadth of the corolla, its angulation and the

position of its lobes, characters good enough were they constantly found in one and never in another. Such, however, is not the case. For instance, the delimited *Echeveria* rests primarily upon its 5-angled corolla, but to *Dudleya* have been assigned species showing this character to some degree. In similar manner, the corolla of *Gormania* is said to be campanulate with spreading lobes, while in *Dudleya* it is tubular and the lobes erect. But in many of the species of *Dudleya* the tips of the lobes, at any rate, are spreading, while corollas occur in both to which the expression short-tubular or narrowly-campanulate might be applied with equal appropriateness. Thus, it appears that through the corolla of *Gormania*, at least, we are back to typical *Echeveria*. Since the characters thus overlap, it would seem best to refer all the species to the oldest genus, in accordance with which view some of the representative ones are here transferred.

*ECHEVERIA* DC.—*Gormania* Britton, Bull. N.Y. Bot. Gard. 3:29. 1903; *Dudleya* Britton and Rose, *loc. cit.* 12; *Cotyledon* L. as to Am. Auth.; *Sedum* L. in part.

*Echeveria Watsonii*, n. comb.—*Gormania Watsonii* Brit. Bull. N.Y. Bot. Gard. 3:29. 1903; *Cotyledon oregonensis* Wats. 1882; not *Sedum oreganum* Nutt.

*Echeveria obtusata*, n. comb.—*Sedum obtusatum* Gray, Proc. Am. Acad. 7:342. 1868.

*Echeveria debilis*, n. comb.—*Sedum debile* Wats. Bot. King's Exp. 102. 1871.

*Echeveria oregana*, n. comb.—*Sedum oreganum* Nutt., in T. and G. Fl. 1:559. 1840.

*Echeveria Gormanii*, n. n.—*Gormania laxa* Britton, Bull. N.Y. Bot. Gard. 3:29. 1903; not *Echeveria laxa* Lindl. 1849.

*Echeveria Brittonii*, n. n.—*Gormania Hallii* Britton, Bull. N.Y. Bot. Gard. 3:29. 1903; not *Dudleya Hallii* Rose, Bull. N.Y. Bot. Gard. 3:17. 1903.

*Echeveria Hallii*, n. comb.—*Dudleya Hallii* Rose, *loc. cit.*

*Echeveria Rusbyi*, n. comb.—*Cotyledon Rusbyi* Greene, Bull. Torr. Bot. Club 10:125. 1883.

*Echeveria saxosa*, n. comb.—*Cotyledon saxosum* Jones, Contr. West. Bot. 8:28. 1898.



*Echeveria nevadensis*, n. comb.—*Cotyledon nevadensis* Wats.  
Bot. Cal. 1:212. 1876.

*Echeveria plattiana*, n. comb.—*Cotyledon plattiana* Jepson, Fl.  
West. Middle California 267. 1901.

*Echeveria Palmeri*, n. comb.—*Cotyledon Palmeri* Wats. Proc.  
Am. Acad. 14:292. 1879.

*Echeveria Rosei*, n. n.—*Echeveria Palmeri* Rose, Bull. N.Y.  
Bot. Gard. 3:10. 1903; not *E. Palmeri* (Wats.) Nels. and Macbr.

*Echeveria lingula*, n. comb.—*Cotyledon lingula* Wats. loc. cit.  
293.

*Echeveria Cotyledon*, n. comb.—*Sedum Cotyledon* Jacq. Eclog.  
1:27. 1811.

*Echeveria Setchellii*, n. comb.—*Cotyledon laxa Setchellii*  
Jepson, Fl. Middle California 267. 1901.

*Echeveria Jepsonii*, n. n.—*Cotyledon caespitosa paniculata*  
Jepson, loc. cit.; not *Echeveria paniculata* Gray, Pl. Wright. 1:76.  
1852.

*Aster siskiyouensis*, n. n.—*Eucephalus glabratus* Greene, Pitt.  
3:56. 1896; not *Aster glabratus* Kuntze.

*Aster perelegans*, n. n.—*A. elegans* T. and G. Fl. 2:159. 1842;  
not *A. elegans* Willd.

*Aster kootenayi*, n. n.—*A. Cusickii Lyallii* Gray, Syn. Fl.  
1:195. 1884; not *A. Lyallii* Kuntze.

*Chaenactis Mainsiana*, n. sp.—Low tufted perennial from a  
slender woody caudex, more or less branched upward: stems few,  
decumbent, naked-pedunculate above: leaves clustered toward the  
base, 3-5 cm. long, including the slender winged petiole, oblanceo-  
late to obovate in outline, once or rarely twice pinnatifid or parted  
into blunt oblong or spatulate lobes, greenish-gray with a minute  
lepidote tomentum, sprinkled with resinous atoms: peduncles  
floccosely-pubescent, exceeding the leaves by 5-10 cm., bearing one  
or often two heads and then subtended by an entire or parted  
bract: heads 1-1.5 cm. high: bracts oblong, obtuse, sometimes  
with one or two shorter and spreading ones: pappus-paleae oblong,  
obtuse, more than half the length of the flower: achene brown,  
sparingly soft-pubescent, about 6 mm. long, distinctly longer than  
the pappus.

This is allied to *C. nevadensis* (Kell.) Gray and *C. Evermannii* Greene, but both of these are dwarf alpine plants with monocephalous peduncles scarcely exceeding the leaves, which show a tendency to be more or less pedately parted and flabelliform. In both the leaves are white with tomentum (at least when young), and the achenes are densely silky-pubescent.

Dedicated to the Supervisor of the Payette National Forest, G. B. MAINS, who is each year adding much to the knowledge of his district by the excellent specimens prepared by him and his associates. The type is no. D-34, from the Payette National Forest.

**Tonestus linearis**, n. sp.—Branches of caudex very slender, brown with remnants of dead petioles: leaves narrowly linear, slightly broadened upward, subacute, 3-nerved, minutely crisped-pubescent, 2-4 cm. long (including the petiole-like base): stems very slender, few-leaved, about 10 cm. long: heads as high as broad; bracts linear, about 10 mm. long, subequal, softly herbaceous, with narrow scarious margins, in two rows, with one or more looser outer ones: rays 10-15, broadly linear, a half longer than the disk; disk flowers more numerous: achene narrowly oblong-cylindric, minutely pubescent.

This new species illustrates the generic characters perfectly, yet by its slenderly linear parts throughout is quite distinct from *T. pygmaeus* and *T. Lyallii*.

Secured by G. B. MAINS, Supervisor Payette National Forest, 1912.

**Balsamorhiza rosea**, n. sp.—Low, acaulescent from a short, thick warty root crowned with leaf bases: leaves somewhat cinereous with a rather long fine but stiff pubescence, including the short petioles 4-10 cm. long, irregularly pinnately divided, the broad short blunt divisions coarsely toothed: scapes few, monocephalous, the pubescence longer and looser than that of the leaves, about 1 dm. high, bearing at base a pair of linear acute bracts 1 cm. long, with scarious clasping bases: heads 3-4 cm. broad, the very crowded persistent or very tardily deciduous rose or purplish rays oval, crenately and irregularly few-toothed at the summit, pubescent with long soft hair, especially on the prominent parallel veins beneath and around the short tube: bracts lanceolate, acute, rather loose, the outer shorter, densely ciliate with long glistening soft pubescence, that of the surface somewhat appressed: chaff of the receptacle broadly scarious-margined three-fourths of the length,

the scarious portion fimbriate at its junction with the herbaceous ciliate tip: ray achenes somewhat compressed, carinate on both sides, hirsute near the top, the pubescence shorter and sparser downward, those of the disk similar but glabrous or nearly so except for the ciliate summit.

This is the second member in the section *KALLIACTIS* Gray. No. 568 by J. S. COTTON, from rocky ridges of the Rattlesnake Mountains, Yakima County, Washington, May 8, 1902, is the type.

**Balsamorhiza serrata**, n. sp.—Low, acaulescent, from a thick short tuber-like root: leaves crowded on the small crown, green but appressed pubescent with minute rigid hairs, the short petioles enlarged and sheathing at base, the blades 3–8 cm. long, much exceeding the petioles, strongly veined, ovate, sharply and closely serrate, the cuspidate teeth longest at the middle, diminishing toward the acute tip and the subcordate base: scapes few (1–3), 1–2 dm. high, monocephalous, naked, or sometimes with a pair of opposite long-petioled laciniately cleft or serrate leaflike bracts below the middle, the pubescence spreading, of two kinds, a fine dense indument and a soft long hirsute form which becomes most pronounced at the base of the heads and on the bracts: heads 4–6 cm. broad, the numerous yellow rays about 3.5 cm. long; bracts linear or linear-lanceolate, acute or acuminate, of nearly equal length: chaff of the receptacle little shorter than the flowers: disk achenes very flat, faintly nerved on each side of the low carinate ridge.

Perhaps nearest *B. deltoidea* Nutt. No. 83 by J. B. LEIBERG, from Morrow County, Oregon, May 19, 1894, is the type. Equally representative is his no. 58 from the same county, May 12, 1894.

ROCKY MOUNTAIN HERBARIUM  
UNIVERSITY OF WYOMING, LARAMIE

## CHEMICAL AND PHYSICAL CHANGES IN GEOTROPIC STIMULATION AND RESPONSE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 179

EVA O. SCHLEY

(WITH SIX FIGURES)

Considerable work has been done on chemical-physical changes involved in tropic presentation and reaction. KRAUS (1) was probably the first worker in this field. His researches include the determination of (a) the sugar content of the growing shoot, (b) the relation of the sugar maximum to the growth maximum, (c) acidity in the growing shoot, and (d) steps in the change of the cell-content of the two halves (the shoot was split longitudinally into the concave half, the inside of the curved portion, and convex half, the outside of the curved portion, of the responded organ) of the shoot exposed to geotropic or heliotropic stimuli during perception and reaction time. He found that (a) the sugar in the growing shoot increases for a certain region from above downward and then decreases, (b) the sugar maximum lies below the growth maximum and consequently is not the limiting factor in growth, (c) the acidity is greatest in the tip and decreases downward. In geotropically and heliotropically stimulated shoots he found, first, decreased acidity and increased sugar on the convex side, later, an increase of water and decrease of sugar on the convex side, followed by a decrease of acidity on this side.

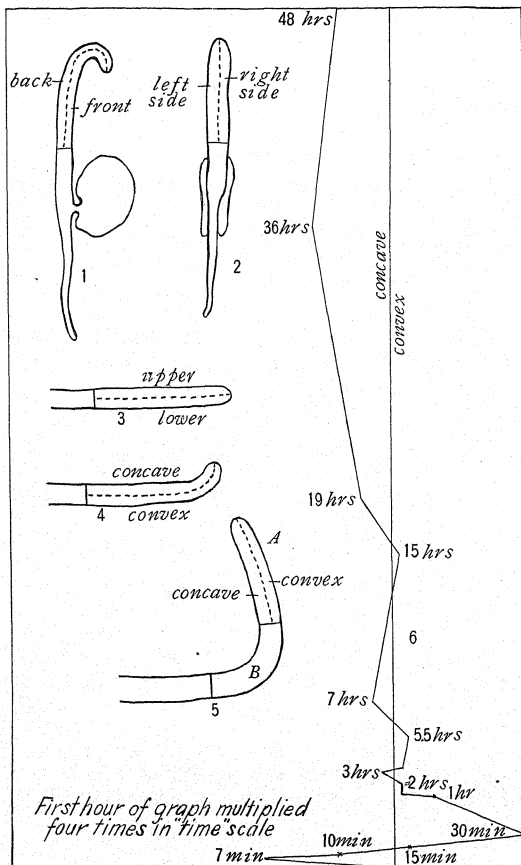
CZAPEK (2) working with geotropically stimulated roots found an interference with the oxidation of tyrosin and phenylalanine which led to the production of homogentisic acid. GROTTIAN (3) and GRAFE and LINSBAUER (4) were unable to confirm his results. MARTIN FISCHER (5) has shown that the water-absorption power of colloids is increased by the addition of acids and alkalies up to a certain concentration, and that salts decrease this power. PROMSY (6) has found that organic acids increase the growth rate of seedlings. RAVIN (7) likewise found that acids increase the rate of

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elongation in growing plant organs. ECKERSON (8) has shown that acids decrease the period of after-ripening of certain dormant seeds. The activity of these acids on germination is due in part to the transformation of zymogens into active enzymes and to favoring the activity of those enzymes. In both germination and growth it is probably partly due to increasing the water-absorbing power of the colloids, especially of the protoplasm.

In view of the effect of acidity on the absorption of water and on the growth rate, it was thought best to reexamine the two halves (concave and convex) of geotropically stimulated and responding organs for difference in acidity, and incidentally for other features such as the sugar content. Following FISCHER's suggestion, it was thought that relative growth on the two sides might parallel the acidity. A later work will examine into this condition of tropic and nastic responses, and a later paper will relate the data more fully to the literature.

Etiolated seedlings grown upright on boards under a spray have been used throughout the experiment. The temperature maintained was approximately 16° C. When the seedlings were 6-8 cm. high, they were used in making tests of acidity upon either the unstimulated or the stimulated organ. For the latter tests the board containing the seedlings was turned on the side so that the seedlings were brought at right angles to gravity. Tests for acidity were made at various intervals of presentation and reaction time, ranging from 7 minutes to 48 hours. In making the tests on the unstimulated shoots, the vertical stem was split longitudinally into right and left halves (in the plane of the cotyledons), and also into back and front halves (at right angles to the plane of the cotyledons) (figs. 1 and 2). For the tests on the geotropically stimulated seedlings the shoots were split longitudinally into upper and lower halves, the former being the half away from the direction of the stimulus and the latter the half next the direction of the stimulus. After reaction these showed as concave and convex halves respectively. These terms hold throughout the paper (figs. 3 and 4). The terminal 4.5 cm. was used, since this region included the region of curvature and since tests on acidity in successive centimeters from the tip downward showed the maximum acidity to be included



FIGS. 1-6.—Explanation in text

in this portion. The cut seedlings were weighed in weighing-bottles and the weight obtained by difference. Samples of 6-8 gm. were used.

Immediately after weighing, the samples were cut up fine and ground to a pulp in a mortar. Several methods of titration were used. In the first method employed, about 50 cc. of distilled water were added to the ground-up tissue and the sample titrated at once without filtration, using phenolphthalein or neutral red as indicator. The objection to the method is that reaction between the acid yet in the tissue with the alkali is slow yet continuous, so that the end point is not definite. A second method used was the test plate method. The tissue was ground as before, filtered through glass wool, and the filtrate refiltered through asbestos over a filter pump. The tissue together with the glass wool filter was again triturated and washed quantitatively into the filtrate previously obtained and the volume made up to 100 cc.; 10 or 25 cc. were used in a titration and the average of several titrations used in calculation. The end point was determined by means of phenolphthalein in the following way. Two solutions of phenolphthalein were prepared. The first was prepared by adding 10 drops of an alcoholic solution of phenolphthalein to 25 cc. of distilled water. The second solution contained in addition 3 drops of a  $n/20$  solution of NaOH, enough to make the solution a decided pink color. A few drops of each solution were placed on a test plate and the sample being titrated tested by introducing a small quantity on the end of a small stirring rod into each of the solutions of phenolphthalein. The end point was reached when the sample introduced just failed to neutralize the small amount of alkali in the one solution, as indicated by the faint pink color remaining, and just showed a faint pink tinge in the other solution. In this way the end point was not obscured by the color of the solution. The method finally adopted was that of using the natural indicator in the plant itself. It has been observed in the course of both animal and plant tissue analysis that chromogens develop when certain fractions, as the lipid fraction, are neutralized. This suggested the idea that possibly the raw material upon neutralization might show a change of color which could be used as an indicator. The reliability of the natural

indicator was tested on several different plants. The results of the experiment are given elsewhere in this paper.

In the case of *Vicia Faba* the material was prepared as in the test plate method; 10-25 cc. of the solution were placed in a porcelain dish and titrated with a  $n/10$  solution of sodium hydroxide. The solution was at first grayish opalescent. It gradually changed during titration, assuming a yellowish color. Upon neutralization the solution turned the color of the testa of the ripe *Vicia Faba* seed. Excess of sodium hydroxide does not change the color. Upon standing 15-30 minutes, however, the neutralized solution becomes dark brown.

The work on acidity included (a) tests on the region of greatest acidity in the shoot, (b) tests on the right and left halves and back and front halves of the unstimulated shoot, and (c) tests on the upper and lower (concave and convex) halves of the geotropically stimulated shoots. The following table will give illustrations of the data obtained in experiments *a* and *b*. The first table gives

TABLE I

*Vicia Faba* shoots in 0.5 cm. lengths from tip downward

1st 0.5 cm. ....	1.30 cc. per g. wt.
2d 0.5 cm. ....	1.25 cc. " " "
3d 0.5 cm. ....	1.07 cc. " " "
4th 0.5 cm. ....	0.84 cc. " " "

TABLE II

*Vicia Faba* SHOOTS; TERM. 4.5 CM. USED

Right half	Left half	Front	Back
0.77	0.71	0.91	0.92
0.88	0.88	0.84	0.83
0.89	0.89	0.98	0.94
0.70	0.67	0.77	0.78
Av. 0.80	0.78	0.87	0.87

results for tests on acidity in 0.5 cm. lengths from the tip downward. The second table shows the acidity of the unstimulated shoots cut longitudinally into right and left halves and front and back halves (figs. 1 and 2). The results as given in table I show decreased acidity from the tip downward, which accords with the work of



KRAUS. Table II is given merely to show the probable error in titration in the following acidity tests of the stimulated shoots.

Table III shows results of titration of the geotropically stimulated shoots at varying intervals of time corresponding to the periods of maximum acidity and to the period of equal acidity of the concave and convex halves.

TABLE III

*Vicia Faba* SHOOTS GEOTROPICALLY STIMULATED; TERMINAL 4.5 CM. USED  
NO. CC. PER GRAM FRESH WT.

Time stim.	Concave half	Convex half	Diff. in acidity
7 minutes.....	0.942	0.715	0.237
10 ".....	0.622	0.469	0.153
15 ".....	0.623	0.683	0.060
30 ".....	0.900	1.210	0.300
60 ".....	1.07	1.19	0.13
2 hours.....	1.01	1.02	0.01
17 ".....	0.512	0.515	0.03
38 ".....	0.884	0.782	0.102
48 ".....	0.671	0.589	0.072

From the tables it will be noticed that the geotropically stimulated shoot first increases in acidity in the upper (concave) half, the observed maximum being 7 minutes. After the maximum the acidity of the concave side rapidly diminishes, passing a period of about equal acidity in 15 minutes. The convex side more slowly approaches a maximum acidity, reaching it in 30 minutes. After the maximum the two sides gradually become equal in acidity until at the time of visible response, about two hours, they are practically equal. This equality in acidity continues through the period of curvature till the plant has passed the vertical, when the concave side again becomes more acid (for these titrations only the curved portion which had passed the vertical plane were used; fig. 5, A). As the shoot straightens again, the acidity decreases on the concave side. The accompanying graph (fig. 6) gives the average of several experiments for each time indicated.

Since this method measures the titration value but not the H-ion content of the acid, the results are not directly comparable with the work of FISCHER, whose conception, besides the acid change, involves also changes in the amount of salts and the nature

of the colloids, which have not been undertaken in this work. So far as the results indicate, however, they show no difference which would explain curvature on the basis of increased acidity, since at the time visible curvature begins the two flanks are of equal acidity.

In testing the natural indicator of the plant, the tissue was prepared as described above for *Vicia Faba*; 25 cc. of the filtrate were used in titration and several duplicate titrations made. Table IV serves to show the accuracy of the titration and to give the color reaction.

TABLE IV

Tissue used	No. cc. $\frac{N}{10}$ NaOH to neut. 25 cc. sol.			Color unneut. sol.	Color neut. sol.
Mucor (young) . . . .	0.13	0.13	0.12	Faintly greenish	Color ripe spores
Wax beans . . . . .	0.36	0.36	0.40	Opalescent	Pale canary yellow
Ripe apple . . . . .	1.61	1.65	1.60	Pale cider	Dark orange
Lemon juice . . . . .	14.80	14.75	14.75	Lemonade	Color lemon rind
Lemon rind . . . . .	0.26	0.23	0.28	Lemon rind	Orange rind
Orange juice . . . . .	2.12	2.18	2.14	Dilute orange juice	Orange rind
Orange rind . . . . .	0.29	0.30	0.30	Pale orange	Deep orange
Etiolated seedlings—					
Corn . . . . .	0.46	0.46	0.44	Faintly yellow	Orange yellow
Sweet peas . . . . .	0.40	0.41	0.40	Opalescent	Canary yellow
Sunflower . . . . .	0.23	0.23	0.20	Dull brownish yellow	Bright greenish yellow
<i>Vicia Faba</i> . . . . .	0.71	0.72	0.70	Faintly grayish	Color ripe seed

Neutral red and phenolphthalein were used with the test plate as checks in determining the accuracy of the indicator. The neutral point is reached when a drop of the alkali fails to produce a change of color in the solution. It is interesting to note that the color produced in neutralization is usually the color of some part of the seed or plant. In *Vicia Faba* this similarity is striking. Solutions of the triturated coat are neutral. The chromogens produced in all of the solutions neutralized were precipitated in 24-48 hours.

Only one sugar titration has been made. For this analysis the etiolated seedlings were grown in sand in the greenhouse at a temperature of about 16° C. When the seedlings were 6-8 cm. high, they were geotropically stimulated for 30 minutes and the terminal 4.5 cm. used in the analysis. The gathering required

half an hour, so that the average stimulation was 45 minutes. After weighing, the two halves (upper and lower) were placed in 85 per cent alcohol heated to 70° C., and allowed to stand for two weeks. The material was then cut up fine and extracted 48 hours in an extractor similar to the Soxhlet extractor. The extract together with the alcohol used in preserving the material was evaporated to moist dryness over the steam bath and brought to constant weight *in vacuo*. The dried material was dissolved in water, 10 cc. of concentrated hydrochloric acid and 20 cc. of chloroform added, and the whole made up to 1000 cc. in volume. The water-soluble portion was filtered from the chloroform-soluble or lipoid fraction and the sugar determination made upon it directly. The lipoid fraction was dissolved in alcohol, and made up to 500 cc. in volume; 50 cc. of each fraction was used in the determination. The fractions were hydrolized and the tannin precipitated before testing for sugar. The method of hydrolysis used was that described in *Bulletin no. 107*, p. 41, U.S. Dept. of Agric., Bur. of Chem. The tannin was precipitated after hydrolysis by means of lead acetate and sodium sulphate. The sugar was determined by the cuprous oxide method as described in the above mentioned bulletin (p. 242). The amount of cuprous oxide was determined by the volumetric potassium permanganate method (*ibid.* pp. 52, 53). An *n*/20 solution was used. The calculations were based on the mg. of copper oxidized in the change from cuprous to cupric oxide, and the invert sugar equivalent obtained from the accompanying table (p. 243). Table V shows the results obtained.

TABLE V

Flank	Fresh wt.	Dry wt.	Amt. sugar	Percentage fresh wt.	Percentage dry wt.
Concave.....	231.635	11.2994	2.7763	1.198	24.50
Convex.....	242.275	11.8064	1.9202	0.792	16.26

These results differ from those of KRAUS in point of time of stimulation. He found the sugar content on the convex side to increase during the period of one hour and then to decrease. The experiment given shows an increase of 0.406 per cent of the fresh

weight and 8.24 per cent of the dry weight on the concave side of seedlings stimulated 45 minutes.

The dry weight of the curved portion of the shoot (fig. 5, B) was determined for various times of stimulation. The material was split into concave and convex halves, weighed, and dried to constant weight in an electric oven at a temperature of  $104^{\circ}\text{C}$ . The differences obtained, while slight, are significant in that they vary in the same direction. Table VI explains itself.

TABLE VI

Time stim. days	Fresh wt. concave grams	Fresh wt. convex grams	Dry wt. concave grams	Dry wt. convex grams	Percentage dry wt. concave	Percentage dry wt. convex	Diff.
2.5.....	22.0139	26.6482	1.2480	1.4234	5.44	5.34	0.09
3.0.....	17.0296	20.8236	.9202	1.0746	5.43	5.16	0.17
4.0.....	54.1735	62.2810	2.8621	3.1524	5.20	5.06	0.14

### Summary

1. The acidity of the growing shoot is greatest at the tip and decreases downward.

2. The relative acidity of the two flanks of the geotropically stimulated shoots changes during presentation and reaction time. First the concave side becomes relatively more acid, then decreases until the maximum acidity comes to lie on the convex side. The two flanks now gradually become equal in acidity, this period coinciding with the time of visible curvature. This equality in acidity is maintained until the tip of the shoot has passed the vertical plane, when the concave side again becomes more acid. As the shoot straightens, the difference in acidity decreases.

3. The increase of acidity does not parallel the relative rate of growth on the two flanks.

4. Several plants examined develop in neutral solution a chromogen which acts as a delicate acid-alkali indicator.

5. The percentage of dry weight is greatest on the concave side.

The writer is indebted to Dr. F. C. KOCH for suggestions in the method of sugar analysis.

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## STUDIES IN THE GENUS *BIDENS*. I

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 180

EARL E. SHERFF

In the prosecution of taxonomic researches upon the different species of *Bidens*, the writer has found several species either apparently undescribed heretofore or bearing names no longer tenable. This article will deal mainly with some of these cases. A list of the various herbaria and libraries consulted thus far, and of the several botanists who have kindly rendered assistance in this work, will be deferred until a later time.

***Bidens Deamii***, sp. nov.—Herba annua, plus minusve pallida, 3 dm. alta, caule profunde ramoso; ramis subglabris, subtetragonis, striatis, inferioribus arcuato-adscendentibus, ramulis monocephalicis. Folia opposita, petiolata, petiolo adjecto 1–2 cm. longa, tri-(aut pinnati-)partita, subglabra; foliolis terminalibus trilobatis, cuneatis; foliolis lateralibus integris aut dentatis; dentibus et lobis submucronatis, integris. Petioli 3–8 mm. longi, submarginati, hispidi-ciliati. Capitula terminalia, longe pedunculata, subcorymbosa, pedunculis adscendentibus aut erectis, 1.5–2 cm. lata, ligulata. Involucrum basi hispidum; squamis duplici serie dispositis; exterioribus linearibus, subviridibus, ciliatis; interioribus paulo longioribus, lanceolatis, margine diaphanis. Ligulae albae aut subroseae, striatae, 8–12 mm. longae. Achaenia linearia, sulcata, spinulosa, biaristata aristis retrorsum hamosis, nigra aut versus apicem fusca, interiora demum plus minusve attenuato-rostrata et 1.2 cm. longa.

*Chas. C. Deam*, Cholula, Mexico, January 1, 1899 (type in Herb. Field Mus.); *Rose, Standley*, and *Russell* 13405, in vicinity of San Blas, Sinaloa, Mexico, March 24, 1910.

A species approaching, in its sometimes slightly beaked achenes, the genus *Cosmos*, but otherwise showing stronger affinities with *Bidens*.

***Bidens parvulifolia***, sp. nov.—Herba caule adscendente aut plus minusve repente, subsimplice, longo 1.5 dm. plusve, subpiloso aut subglabro, tetragono. Folia opposita, petiolata, ovata, acuta  
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aut subacuminata, serrata (aut summum jugum trifoliatum lobis serratis), pubescentia, petiolo adjecto 1.5–2.5 cm. longa. Petioli 6–8 mm. longi, ciliati, pilosiusculi. Capitula terminalia, longe pedunculata, 3 cm. lata. Involucrum basi hispidulum, squamis duplici serie dispositis; exterioribus linearibus, glabris; interioribus longioribus, lanceolatis, margine diaphanis. Ligulae flavae (in specim. exsicc.), striatae, 1.2 cm. longae. Achaenia matura absentia. Ovaria biaristata, aristis retrorsum hamosis.

*Heyde and Lux* 6163, Guatemala, 1894 (type in Herb. Univ. Chicago); *eidem* 6162, Fraijanes, Depart. Amatitlan, Guatemala, alt. 900 m., September 1893.

***Bidens ramosissima***, sp. nov.—Herba annua, pallida, ramosissima, 3–5 dm. alta; ramis tenuibus, oppositis, sulcatis, subglabris aut hispidis; ramulis monocephalicis. Folia petiolo adjecto 8–15 mm. longa, tri-(aut pinnati-)partita; foliolis integris aut trilobatis, 1–7 mm. longis, acutis, base angustis aut cuneatis; petiolis 2–4 mm. longis, ciliatis et nonnullis hispidis. Capitula terminalia, subcorymbosa, longe et tenuiter pedunculata, ligulata, 1–2 cm. lata. Involucrum basi hispidum, demum reflexum, squamis duplici serie dispositis; exterioribus linearibus, ciliatis, apice plus minusve dilatatis, mucronatis, 3 mm. longis; interioribus subglabris, lanceolatis, margine diaphanis, 4–5 mm. longis. Ligulae albae aut apice subviolaceae, obovatae, striatae, truncato apice 3–5 dentato dentibus obtusissimis, 6–8 mm. longae. Paleae lineares, 4 mm. longae. Achaenia linearia, sulcata, biaristata, margine et lineis mediis scabrido-hispida, 8–10 mm. longa; aristis retrorsum hamosis.

*W. E. Safford* 1391, near Guadalajara, Jalisco, Mexico, February 23, 1907 (type in U.S. Nat. Herb.).

A species suggestive of *B. Deamii*, but differing in the finally reflexed involucre and the more slender, highly subdivided branches. It is one of the several species of *Bidens* that approach too closely, in color of rays or beaked achenes, the genus *Cosmos*. However, distinctly rostrate achenes are absent in the type, and the involucre, furthermore, is that of a true *Bidens*. On label said to have the common name "accitillo."

***Bidens mexicana***, sp. nov.—Herba caule scandente, ramoso, tereti, striato, glabro. Folia opposita, petiolata, pinnati-(aut bipinnati-)divisa; foliolis aut laciniis submembranaceis, serratis,

glabris aut subciliatis; lateralibus petiolulatis aut subsessilibus, lanceolatis aut ovato-lanceolatis, attenuatis, 2-6 cm. longis; terminale plus minusve petiolulato, lanceolato-attenuato, apice anguste acuminato, basi subcuneato, 4-6 cm. longa. Petioli 1.5-4 cm. longi, glabri, angusti, basi dilatati et connati. Capitula plurima, corymbosa aut paniculato-corymbosa, pedicellata, 6-7 mm. alta, 5-7 mm. lata. Involucrum squamis duplici serie dispositis; exterioribus linearibus, glabris, squarrosis aut reflexis, 3 mm. longis; interioribus lanceolatis, striatis, margine diaphanis, glabris aut apice minute pubescentibus, 4-5 mm. Ligulae flavae, striatae, 8-10 mm. longae. Paleae lineare-lanceolatae, striatae, glabrae aut apice subglabrae, 5 mm. longae. Achaenia nigra, biaristata, margine tuberculato-hispida, 7-9 mm. longa, aristis squarrosis aut recurvis.

*Dr. Edward Palmer* 95, in or near Acapulco, Mexico, October 1894 to March 1895 (type, with the flowering and the fruiting specimen on two separate sheets, in Herb. Univ. Chicago).

An interesting species, allied to *B. reptans* (L.) G. Don and *B. tereticaulis* DC., but apparently related even more closely to *B. Urbanii* Greenman. Various specimens of this number elsewhere in the United States show a considerable range of variation in leaf texture and outline; but the flowering type specimen exhibits leaves strikingly close to those of *B. Urbanii* Greenman (see *B. dissecta*, below), of which this species may be the Mexican representative.

***Bidens Brittonii*, sp. nov.**—Herba caule scandente, striato, ramis oppositis, 2-4 m. alto. Folia petiolo adjecto 3-9 cm. longa, pinnata; foliolis dentatis incisus aut pinnatisectis, supra pubescentibus, subtus tomentosis, 0.8-2 cm. longis; petiolis et mediis nervis ciliatis. Capitula corymbosa, breviter pedunculata, ligulata. Involucrum 4-6 mm. altum, 5-6 mm. latum, squamis inter se subaequalibus, duplici serie dispositis; exterioribus lineari-spatulatis, ciliatis, nonnullis pubescentibus; interioribus linearibus, apice pubescentibus et angustioribus, margine diaphanis. Ligulae flavae, anguste obovatae, non apice dentatae, 1.5 cm. longae. Paleae lineares, apice pubescentes, 6-8 mm. longae. Achaenia linearia, striata, biaristata, margine tuberculato-hispida.

*C. Wright* 314 *pro parte*, eastern Cuba, 1856-1857 (type in Gray Herb.).

The achene characters are drawn from a fruiting specimen (also collected by *WRIGHT*, 314) in the Columbia College Herbarium. This specimen, as also



the one at the Gray Herbarium, was mounted upon the same sheet with a specimen of *B. reptans* (L.) G. Don. On the sheet was written, evidently in TORREY'S handwriting, "2 species!"; while just below, GRAY had written, "no, A. Gr." In his diagnosis of WRIGHT'S plants, GRAY treated this form as a variety of *B. reptans* with dissected leaves. But the fact that the leaves are pubescent above and strongly tomentose beneath, as well as finely dissected, makes it seem certain that two different species have been confused, and that TORREY'S assumption was correct. The species is here named in honor of Dr. N. L. BRITTON in grateful recognition of his assistance, upon this and several related species, in supplying data from his widely extended field knowledge of West Indian plants.

***Bidens dissecta***, comb. nov.—*B. reptans* (L.) G. Don, var. *dissectus* O. E. Schulz, Urb. Symb. Antill. 7<sup>1</sup>: 142. 1911.

A Jamaican plant similar to the Porto Rican *B. Urbanii* Greenman, but with more finely divided leaves; also to the Cuban *B. Brittonii*, but with the more finely divided leaves not tomentose beneath. These three species, while perhaps congeners at a remote period in the past, and certainly related to *B. reptans* (L.) G. Don, are, in my opinion, clearly entitled to specific rank and should be classed as separate species. They seem quite distinct from even the most closely approaching forms of the highly variable *B. reptans* (L.) G. Don, many specimens of which I have seen at the New York Botanical Garden, through the courtesy of Dr. BRITTON.

***Bidens anthemoides***, comb. nov.—*Coreopsis anthemoides* DC. Prodr. 5: 573. 1836.

ASA GRAY (Proc. Amer. Acad. 19: 15. 1884) carefully considered this species, but retained it in *Coreopsis*, the taxonomic distinctions between which and *Bidens* he admitted frequently in his writings to be highly artificial. But since the days of DE CANDOLLE and GRAY, botanists have very correctly inclined toward separating these two genera according to the sum total of the characters of each species. In the several transfers consequently made, notably by BRITTON (Bull. Torr. Bot. Club 20: 280-281. 1893), this and the following species seem to have been overlooked. It is interesting to note, however, that C. H. SCHULTZ BIPONTINUS had given, previous to GRAY'S observations, the names *Bidens coreopsidioides* (not *B. coreopsidis* as in Gray, loc. cit.) and *Bidens Schaffneri* to specimens of this species sent to the Gray Herbarium.

***Bidens Schaffneri***, comb. nov.—*Coreopsis Schaffneri* A. Gray, Proc. Amer. Acad. 19: 15. 1884.—A perennial species allied with *B. angustissima* H. B. K., *B. procera* Don, and even *B. ludens* Gray.

Evidently placed in *Coreopsis* by GRAY merely because of the practically smooth awns of the achenes. In general habit, also in the shape of the achenes

(which are tetragonal and lack wings), GRAY's type is a true *Bidens*. The associate types collected by PARRY and PALMER (488 and 488½, not "448 and 448½" as erroneously printed in GRAY's original citation), in the Gray Herbarium, match very well SCHAFFNER's no. 202, the type. The name here given for this species should not be confused with the same name given years ago by SCHULTZ BIPONTINUS to *B. anthemoides* on a herbarium label, but never published by him.

*BIDENS TENUISSIMA* Greene, Leaf. Bot. Crit. 1:200. 1906.—This species, termed *Bidens tenerrima* on the labels of the type collection, was originally described by GREENE as "a gigantic ally of *B. connata* but with almost minute heads." And indeed, when the specimens are compared with the true, ternate-leaved *B. connata* Muhl., the difference in the heads is striking. On carefully comparing them, however, with tall slender forms of *B. discoidea* (T. & G.) Britton, I am unable to find any specific difference. GREENE's type, also the several excellent cotypes in various United States herbaria, should be retained as merely tall, slender forms of *B. discoidea* (T. & G.) Britton.

*BIDENS DAHLIOIDES* Watson, Proc. Amer. Acad. 26:142. 1891.—At times bearing only rostrate achenes and in this respect simulating *Bidens*, to which genus it had already been referred under different names by earlier writers. But from the involucre, the ligules, and the frequently occurring rostrate achenes, it is seen to be a true species of *Cosmos*. As such it was described long before by OTTO, under the name *Cosmos diversifolius*, and one may well conclude that WATSON merely overlooked OTTO's species by mistake.

*BIDENS INCISA* (J. B. Ker) G. Don.—*Coreopsis incisa* J. B. Ker, Bot. Reg. 1:7. 1815; *Bidens incisa* G. Don, Sweet Hort. Brit. ed. 3:360. 1839; *Bidens reptans* (L.) G. Don, var. *tomentosus* O. E. Schulz, Urb. Symb. Antill. 7:141. 1911.

KER very justly separated this species from the smooth-stemmed *B. reptans* (L.) G. Don. He laid too much stress, however, upon the "indented ray" of the latter species, this character varying too much to be of certain value. The recently described *B. reptans tomentosa* O. E. S., based on GEO. E. NICHOLS' no. 137, is merely a slightly smaller-leaved form of *B. incisa* (L.) G. Don, the terminal leaflets being less than "about two inches long." A study of several other collectors' specimens, notably at the New York Botanical Garden, some of which Dr. BRITTON had already labeled *Bidens incisa*, showed

that the terminal leaflet varies in length from about 5 cm. to less than 3 cm. Some of the specimens examined were as follows: *J. H. Hart* (without date); *Geo. E. Nichols* 137, particularly the specimen in the Mo. Bot. Gard. Herb.; *E. G. Britton*, N.Y. Bot. Gard. Explor. Jam. 3867; *N. L. Britton*, same series 73.

***Bidens coronata* (L.) Britton, auctor emend.—*B. coronata* (L.) Fischer, Britton in Bull. Torr. Bot. Club 20:281. 1893.**

BRITTON, at the time of transferring several American species from *Coreopsis* to *Bidens*, assumed that this species had already been transferred by FISCHER. In fact, as his citation proves, he rested his assumption entirely upon STEUDEL. But a careful examination of STEUDEL (Nomencl. Bot. ed. 2. 1840), with proper regard for his use of italics for synonyms, shows that FISCHER's plant was *Coreopsis coronata* Hooker (a true *Coreopsis*) and not *Coreopsis coronata* L. STEUDEL even emphasized this fact with the words *nec alior* after *C. coronata* Hooker, to which he referred *Bidens coronata* Fischer. Thus the Linnean species was left in *Coreopsis* until BRITTON's treatment of it as a species of *Bidens*. That the name may be given a definite and technically correct status, it is here set forth as *Bidens coronata* (L.) Britton.

UNIVERSITY OF CHICAGO

## SOME ALASKAN LICHENS

R. HEBER HOWE, JR.

(WITH TWO FIGURES)

During the summer of 1911, the late FRED. B. McKECHNIE, who accompanied Mr. A. C. BENT on the U.S. National Museum Expedition to Alaska, collected lichens for me in various localities, mostly on the coast of Alaska. The crustose species I sent to Dr. H. E. HASSE of Santa Monica, Cal., for determination, and the species of *Cladonia* and *Stereocaulon* were kindly named by Dr. L. W. RIDDLE of Wellesley, Mass. I have arranged the species in accord with Miss CLARA E. CUMMINGS' *Lichens of Alaska* (1910) for convenience in comparison, as well as with my Yukon list.<sup>1</sup> Mr. MERRILL's Yukon lichens<sup>2</sup> should not be overlooked among the recent papers on boreal northwest species.

### SPHAEROPHORIACEAE

1. SPHAEROPHORUS (CORALLOIDES) GLOBIFERUS (L.) DC.—Ketchikan, May 27, 1911; Little Kiska Is., June 19, 1911, "1000-1500 ft."; Unalaska Is., Chernofski, June 10, 1911.

### LECIDIACEAE

2. BAEOMYCES (AERUGINOSUS) ERICETORUM (L.) Wain.—Ketchikan, May 27, 1911, "rotten stump."

### CLADONIACEAE

3. CLADONIA RANGIFERINA (L.) Web.—Little Kiska, June 19, 1911, tundra, 1000-1500 ft.; Atka, June 27, 1911, grass, 1500 ft.
4. CLADONIA SYLVATICA (L.) Hoffm.—Little Kiska, June 19, 1911, tundra 1000-1500 ft.; Atka, June 27, 1911, grass, 1500 ft.
5. CLADONIA UNCIALIS (L.) Web.—Amaknak Is., June 6, 1911, moss, 1200 ft.; Nome, July 14, 1911.
6. CLADONIA COCCIFERA (L.) Willd.—Nome, July 13, 1911, meadows.

<sup>1</sup> Bull. Torr. Bot. Club 38:287-293. 1912.

<sup>2</sup> Bryologist 11:105-111. 1908.

7. CLADONIA CORNUTA (L.) Schaer.—Atka, June 27, 1911, 1500 ft.
8. CLADONIA DECORTICATA (Flk.) Spreng.—Atka, June 27, 1911, 1500 ft.
9. STEREOCAULON TOMENTOSUM Fr.—Unalaska Is., Chernofski, June 9, 1911, tundra, 150 ft.
10. STEREOCAULON PASCHALE (L.) Ach.—Atka Is., June 22, 1911, tundra, 1000 ft.

## LECANORIACEAE

11. LECANORA OCCULATA (Dicks.) Ach.—Little Kiska, June 19, 1911; Atka, June 26, 1911.
12. LECANORA SAXATILIS (L.) Schaer (*L. muralis*).—St. Paul Is., June 1911; three specimens.
13. LECANORA CASTANEA (Hepp.) Th. Fr.—Amaknak Is., June 6, 1911, 1200 ft.
14. LECANORA CARTILAGINEA Ach.—St. Paul Is., June 1911.
15. OCHROLECHIA GEMINIPARA Th. Fr.—Nome, July 14, 1911.
16. OCHROLECHIA UPSALIENSIS Nyl.—Nome, July 4, 1911.
17. CALOPLACA CALOPISMA (Ach.) Th. Fr.—Chernofski, June 10, 1911.

## PELTIGERIACEAE

18. PELTIGERA CANINA (L.) Hoffm.—Amaknak Is., Little Kiska Is., June 19, 1911, "tundra, 1000-1500 ft."; June 6, 1911, 1000 ft., "growing on ground just above and below snow line"; Akun Is., June 4, 1911, "dead logs"; Atka Is., June 13, 1911, "tundra"; four specimens, three of which represent the variety *spongiosa* Tuck.

19. NEPHROMA ARCTICUM (L.) Fr.—Nome, June 14, 1911.
20. *Lobaria* (STICTA) *oregana* (Tuck.), comb. nov.—Ketchikan, May 26, 27, 1911, "thick fir growth."

## UMBILICARIACEAE

21. GYROPHORA (UMBILICARIA) ARCTICA Ach.—Atka Is., June 27, 1911, "rocks, 1000 ft., snow near by"; two specimens.

## PARMELIACEAE

22. PARMELIA SAXATILIS Ach.—Unalaska Is., Chernofski, June 9, 1911, "on rocks along the beach," two specimens; St. Paul Is., July 6, 1911, "rocks near beach," one specimen.

23. *PARMELIA SAXATILIS* Ach. var. *SULCATA* (Tayl.) Nyl.—Akun Is., June 4, 1911, "on logs."

24. ———, var. *OMPHALODES* (L.) Fr.—Atka Is., June 27, 1911, "2000 ft. on rocks"; St. Paul Is., July 7, 1911.

25. ———, var. *FURFURACEA* Schaer.—St. Paul Is., July 6, 1911, "on rocks." All these specimens show the dicroic reddish margins mentioned by MACOUN and Miss CUMMINGS.

26. *XANTHORINA* (*TELOSCHISTES*) *LYCHNEA* (Ach.) Th. Fr.—The material represents according to Dr. HASSE the variety *lacinosa* Schaer, but like material was referred to the variety *pygmaea* (Fr.) Th. Fr. by Miss CUMMINGS, St. Paul Is., July 6, 1911, "on rocks."

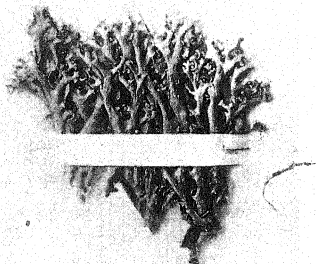


FIG. 1.—The Bellardi-type of *Lichen cuculata* preserved at Turin

#### USNEACEAE

27. *ALECTONIA OCHROLEUCA* var. *CINCEINATA* (Fr.) Nyl.—Unalaska, Chernofski, June 11, 1911, "200 ft."

28. *COELOCAULON* (*ALECTORIA*) *DIVERGENS* (Ach.) Howe.—Little Kiska Is., June 19, 1911, "tundra, 1000-1500 ft."

29. *PLATYSMA* (*CETRARIA*) *LACUNOSUM* (Ach.) Nyl.—Atka Is., June 26, 1911; Ketchikan, May 26-27, 1911, "on fir limb twigs"; Little Kiska Is., June 19, 1911, "1000-1500 ft., tundra." These specimens represent the true *lacunosum* of ACHARIUS (type loc. "America boreali"). Mr. MERRILL renamed the typical form

("rugoso-reticulato celluloso albo subvirescente" Ach.) when he (Bryol. 13:26. 1910) made the forma *cavernosa* (Menzies) Merr. (reticulate-lacunose-cellulose"—"whitish-cinereous" Merr.). The less lacunose virescent examples with perforate apothecia, common in the eastern states, were named by TUCKERMAN as the variety

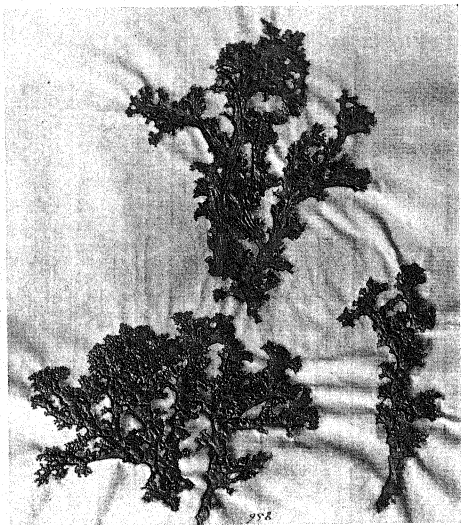


FIG. 2.—The Linnean type of *Lichen nivalis* preserved at London

*atlantica* (Tuck.) Nyl. It is, in view of the true type, a well marked variety, which has been lost sight of lately. *Platysma glaucum* (L.) Nyl. is never lacunose-reticulate above, always nitidous below, and the margins are always crisped.

30. *PLATYSMA* (*CETRARIA*) *GLAUCUM* var. *stenophyllum* (Tuck.), comb. nov.—Ketchikan, May 27, 1911. The margins of

this variety are always *dissected*, never *entire* as in var. *stenophyllum* of the preceding species.

31. CETRARIA CUCULLATA (Bell.) Ach.—Amaknak Is., June 6, 1911.

32. CETRARIA NIVALIS (L.) Ach.—Amaknak Is., June 6, 1911. The plate of the types of the above two species it is thought of value to publish. Dr. WAINIO failed to record the *nivalis* type as represented in the Linnean herbarium. Through the kindness of Dr. B. DAYDON JACKSON of the Linnean Society, I was enabled to photograph it, as also the Bellardi type preserved at Turin in the R. Istituto Botanico through the kindness of Dr. O. MATTIROLLO.

33. DACTYLINA MADREPORIFORMIS (Wolf.) Ach.—Little Kiska Is., June 19, 1911, "1000-1500 ft., tundra."

34. RAMALINA SUBFARINACEA Nyl. (*R. angustissima* [Anzi.] Wain., a nomen nudum).—St. Paul Is., June 6, 1911, "rocks near beach," Atka Is., June 13, 1911, "tundra." These specimens represent evidently the same material mentioned under *Ramalina cuspidata* Nyl. (KOH-) and *Ramalina javanica* Nyl. by Mr. MERRILL (Bryol. 11:51. 1908). The plants are referable to *Ramalina calicaris* (L.) Fr. emend. (*R. scopulorum* [Retz.] Ach.)<sup>3</sup> except for their subterete lacinia, and more or less multified, soresiate tips (KOH+). The smaller examples suggest a little *Ramalina intermedia* Nyl. (KOH-).

THOREAU MUSEUM OF NATURAL HISTORY  
CONCORD, MASS.

<sup>3</sup> See Bryol. 16: Nov. 1913.



## BRIEFER ARTICLES

### INCLUDED CYTOPLASM IN FERTILIZATION

In the recent review<sup>1</sup> of NĚMEC's paper on the fertilization of *Gagea lutea*,<sup>2</sup> the following statements occur: "Another apparently unusual feature is the inclusion of cytoplasm between the fusing nuclei. . . . This is the second record of such a cytoplasmic inclusion, the first having been made by BROWN<sup>3</sup> in his study of *Peperomia*."

Since this is a rarely recorded phenomenon, I may be permitted to call attention to some earlier references to the inclusion of cytoplasm between the sexual nuclei in plants at the time of fusion. In a paper published in 1901,<sup>4</sup> the following sentence occurs (p. 450): "Frequently the cytoplasm caught between the two nuclei collects in spherical masses; between these spheres of cytoplasm the membranes of the nuclei come into close contact (fig. 52)." This sentence is likewise repeated on p. 116 of a later publication.<sup>5</sup> I would also call attention to figs. 51, 53, and 54 of the earlier paper, and figs. 224-227 of the later paper, which were redrawn from the same preparations as the figures published earlier.

BROWN first figured and described the inclusion of cytoplasm between the fusion nuclei in 1908.<sup>6</sup> If other students of plants have mentioned this subject, I am not aware of it.

In the papers published in 1901 and 1904, I made no special comment, other than the sentence quoted above, on the inclusion of cytoplasm between the sexual nuclei, because this is one of many observations which have convinced me that nucleus and cytoplasm cooperate in all cell

<sup>1</sup> CHAMBERLAIN, CHARLES J., Fertilization in *Gagea*. BOT. GAZ. 55:472. 1913.

<sup>2</sup> NĚMEC, B., Über die Befruchtung bei *Gagea*. Bull. Internat. Acad. Sci. Bohême 1912: 1-17. figs. 19.

<sup>3</sup> BROWN, W. H., The exchange of material between nucleus and cytoplasm in *Peperomia sentenisi*. BOT. GAZ. 49:189-194. pl. 13. 1910.

<sup>4</sup> FERGUSON, MARGARET C., The development of the egg and fertilization in *Pinus Strobus*. Ann. Botany 15:435-479. pls. 23-25. 1901.

<sup>5</sup> ———, Life history of *Pinus*. Proc. Wash. Acad. Sci. 6:1-202. pls. 1-24. 1904.

<sup>6</sup> BROWN, W. H., The nature of the embryo sac of *Peperomia*. BOT. GAZ. 46: 445-458. 1908.

divisions, whether in somatic or in reproductive cells. In the description of the division of the generative cell in *Pinus*,<sup>7</sup> this sentence appears (p. 209): "That the spindle-fibers which originate in the cytoplasm and apparently grow by a differentiation of its network are later fed by the linin of the achromatic nuclear reticulum there seems little room for doubt. In fact, all the phenomena connected with this division indicate that we are dealing, not with persistent cell-constituents, but with different manifestations of one and the same thing." With variations in method of expression, this idea is promulgated again and again in the three papers on *Pinus* to which reference has already been made.

It is interesting to find such convincing evidence of the transformation of cytoplasm to nucleoplasm, or at least, of its disappearance in the nucleoplasm, as that given by BROWN. The close student of the phenomena of fecundation and of nuclear division finds; also, many evidences, less clearly demonstrable but equally convincing, of the intimate relation between these two portions of the protoplasm.

There can be no doubt that threads from the cytoplasm unite with portions of the nuclear reticulum to form the spindle-fibers in the division of the generative cell in *Pinus*; and it is equally evident, at the time of fertilization, not only that the "included cytoplasm" disappears in the area occupied by the conjugating nuclei, but that a large part of the reticulum of the egg nucleus disappears in the general cytoplasm. These and similar unpublished observations on other plants convince me that certain portions, at least, of cytoplasm and nucleus are interchangeable.—MARGARET C. FERGUSON, *Wellesley College*.

## HENRY WILLEY

(WITH PORTRAIT)

The work of HENRY WILLEY in lichenology entitles him to a more definite introduction among botanists than he has received. He was born in Geneseo, N.Y., July 10, 1824, and died in South Weymouth, Mass., March 15, 1907. During his active life he was the editor of a newspaper in New Bedford Mass., resigning that position in 1900. As a student of lichens, he probably ranked second only to TUCKERMAN, whose pupil he was, and whose last work he edited. His various contributions to the literature of lichens appeared during the period from 1867

<sup>7</sup> FERGUSON, MARGARET C., The development of the pollen tube and the division of the generative nucleus in certain species of pines. *Ann. Botany* 15:193-223. pls. 12-14. 1901.

to 1892, and then he disappeared from botanical literature during the last fifteen years of his life.

As a man, he was very eccentric, being exceedingly diffident and having only the one great interest. For the most part he lived alone in complete seclusion, with his library and herbarium as his only companions. The herbarium was purchased for the National Herbarium after his death, so that the permanent record of his work has been made available for all subsequent students of lichens. Although a recluse socially, he was very generous in his work for others, determining cheerfully hundreds of specimens of lichens that were sent to him, but brusquely resenting any apparent effort to impose upon him.

The inner flavor of the man may be obtained by the following tribute he paid to TUCKERMAN:

And here I would pay a last tribute to the memory of one to whom, for the unbounded liberality of the information imparted from 1862 to the time of his death, and for his patience under my sometimes too great demands upon him, I am under such great obligations.

EDWARD TUCKERMAN was surpassed by none of the botanists of his day in his disinterested love of truth, in the patience with which he sought to unravel the difficulties of the most difficult of all plants, and in the philosophic spirit with which he labored to trace affinities and to bring them into natural connection.

A very interesting account of HENRY WILLEY, from which the above information was obtained, was published in the *New Bedford Standard* of July 20, 1913, prepared by R. HEBER HOWE, JR.—J. M. C.



Henry Willey

# CURRENT LITERATURE

## NOTES FOR STUDENTS

**Metabolism of fungi.**—Among recent papers on the metabolism of fungi two on protein synthesis are of special interest. The first by EHRLICH<sup>1</sup> relates to the utilization of the nitrogen of amino acids when substances other than sugar form the source of energy. In his former work EHRLICH showed that in the presence of sugar the  $\alpha$ -amido acids are transformed by yeast into carbon dioxide, ammonia, and alcohols with one carbon atom fewer than the corresponding amino acids, the ammonia being used in protein synthesis, while the carbon residue is excreted as a waste product. In the present paper he reports the results of an investigation of the action of yeasts on an amino acid, tyrosin, when simpler compounds such as alcohol, glycerine, lactic acid, etc., are supplied as sources of carbon. He finds that the decomposition of the amino acid proceeds in the same manner regardless of the source of carbon, and that even with these less favorable sources of energy the nitrogenous radical only of the amino acid is utilized by the organism. In cultures containing cane sugar, glycerine, or alcohol in addition to tyrosin, small quantities of esters and volatile fatty acids were formed. *Oidium lactis* was found to behave in an analogous manner. EHRLICH and JACOBSON had shown that in the presence of sugar this fungus produces from amino acids the corresponding oxyacids. Its behavior in this respect was not changed when glycerine, lactic acid, or alcohol were used instead of sugar. With each of these substances tyrosin yielded paraoxy-phenyl lactic acid, only the nitrogenous portion of the molecule being utilized by the fungus.

PURIEWITSCH<sup>2</sup> approached the problem of protein synthesis from another point of view, by determining the energy required, as measured by the carbon dioxide output per unit of dry weight of fungus, for the assimilation of different nitrogenous compounds by *Aspergillus niger*. Of the large number of nitrogenous compounds tested with sugar, low ratios of carbon dioxide to dry weight were obtained with methyl urea, the amino acids, potassium sulphocyanate, acetamide methyl amine, and urea, while such compounds as guanidin, ethylamine, potassium nitrate, peptone, protein, and phenyl urea gave higher ratios, showing that more energy is required for their assimilation. Fewer nitrogenous compounds were tried in connection with succinic acid, malic acid,

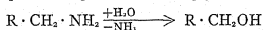
<sup>1</sup> EHRLICH, F., Über die Bildung des Plasmaeiweisses bei Hefen und Schimmelpilzen. Biochem. Zeitschr. 36:477-497. 1911.

<sup>2</sup> PURIEWITSCH, K., Untersuchungen über die Eiweissynthese bei niederen Pflanzen. Biochem. Zeitschr. 38:1-13. 1912.

or tartaric acid in place of sugar. The ratios for these acids were higher than those obtained with sugar, and they increased for the different acids in the order in which the acids are named. The general arrangement of the nitrogenous compounds in the order of their assimilability was essentially the same for these acids as for sugar.

As a result of his experiments, PURIEWITSCH favors the view of CZAPEK, according to which the  $\alpha$ -amino acids serve directly as materials for protein synthesis. He has overlooked, however, much of the recent work of EHRLICH and others who have shown conclusively, for yeasts and *Oidium lactis* at least, that it is only the amido group in the form of ammonia that is used for protein synthesis, while the rest of the molecule is entirely rejected. The author, therefore, is in error when he states that there is no evidence that the ammonia split off from organic compounds serves as a source of material for protein synthesis.

A further contribution to the subject of nitrogen metabolism of fungi has been made by EHRLICH and PISTSCHIMUKA,<sup>3</sup> in their study of the fermentation of the primary amines. They find that with yeasts, especially *Willia anomala* and wild yeasts, and *Oidium lactis*, these substances undergo a fermentation, resulting, like the analogous fermentation of amino acids, in the production of the higher alcohols. The process results in a substitution of the amido group by the hydroxy group according to the following general reaction:



By this process tyrosol was obtained from p-oxyphenylethylamine and fusil oil from isoamylamine. As in the case of the amino acids, only the nitrogenous part of the molecule is utilized by the organisms, the rest of the molecule being transformed into the corresponding alcohol.

The easy fermentation of amines to the corresponding alcohols raises the question whether the transformation of amino acids to alcohols does not take place with the intermediate formation of amines rather as NEUBAUER and FROMHERZ<sup>4</sup> believe, than with the intermediate formation of keto-acids.

In continuation of his studies on nitrogen metabolism, EHRLICH<sup>5</sup> reports a further example of the fermentation of an amino acid with the production of the corresponding alcohol. He obtained tryptophol ( $\beta$ -indolyethyl alcohol) by the action of yeast on tryptophan ( $\beta$ -indolyl alanin). The chemical properties of the new compound are described in detail.

The influence of nutrition on the secretion of enzymes by fungi, a problem concerning which different investigators have arrived at different conclusions,

<sup>3</sup> EHRLICH, F., und PISTSCHIMUKA, P., Überführung von Aminen in Alkohole durch Hefe- und Schimmelpilze. Ber. Deutsch. Chem. Gesells. 45:1006-1012. 1912.

<sup>4</sup> Rev. Bot. GAZ. 54:343. 1912.

<sup>5</sup> EHRLICH, F., Über Tryptophol ( $\beta$ -indolyethyl Alcohol) ein neues Gärprodukt der Hefe aus Aminosäuren. Ber. Deutsch. Chem. Gesells. 45:883-889. 1912.

has been investigated by GREZES<sup>6</sup> with respect to the production of invertase and other enzymes by *Aspergillus niger*; and by KNUDSON with respect to the production of tannase by *Aspergillus niger*, *Penicillium rugulosum*, and an unknown species of *Penicillium*.

GREZES finds that *Aspergillus niger*, even when grown on RAULIN's solution containing no sugar and with succinic acid as the sole source of carbon, produces invertase, diastase, maltase, inulase, and emulsin. A quantitative study of the production of invertase by the fungus on media containing, on the one hand cane sugar and on the other succinic acid, a substance not related to the carbohydrate group, showed that the production of the enzyme is greatly influenced by the mode of nutrition. Only small quantities of invertase are produced by cultures on succinic acid, yet its production is not entirely suppressed, even after 60 spore-generations on succinic acid, and nearly normal production immediately takes place if the fungus is transferred to sugar solutions. The author concludes that the power of invertase production is an inseparable characteristic of the fungus.

KNUDSON<sup>7</sup> finds that tannase is not produced by *Aspergillus niger* nor by the species of *Penicillium* which he studied if there is no tannic acid or its decomposition product, gallic acid, in the solution. If either of these substances is present there is a progressive increase in the production of tannase with increase of concentration of the acids. With a constant concentration of tannic acid the production of the enzyme is depressed with increasing concentration of sugar. Mycelia grown on various organic compounds other than gallic acid contain no tannase. Gallic acid stimulates the production of tannase even in solutions containing 10 per cent of cane sugar.

A number of papers deal with the hydrolysis by fungi of glucosides, tannin, and other less complex compounds. UHLENHAUT<sup>8</sup> studied the hydrolysis of amygdalin by the action of fungi, but, aside from extending somewhat the list of fungi capable of splitting amygdalin, his paper adds nothing essential to that which is already known concerning the process. The 14 species which he investigated differed considerably, as judged by purely qualitative tests, in their capacity for hydrolyzing the glucoside. The mucors were in general found to be more active in this respect than the higher fungi. The growth of the mucors on amygdalin solutions was usually soon inhibited, owing to the accumulation of benzene cyanhydrin, to which the author ascribes the hydrocyanic acid odor developed in the cultures. If, however, other fungi capable of utilizing the excess of cyanhydrin are grown in cultures together with mucors,

<sup>6</sup> GREZES, G., Recherches sur la sucrase de l'*Aspergillus niger*. Ann. Inst. Pasteur 26: 556-573. 1912.

<sup>7</sup> KNUDSON, L., Tannic acid fermentation. II. Effect of nutrition on the production of the enzyme tannase. Jour. Biol. Chem. 14: 185-202. 1913.

<sup>8</sup> UHLENHAUT, H., Über die Spaltung des Amygdalins durch Schimmelpilze. Ann. Mycol. 9: 567-621. 1911.

the mucors flourish better than in pure cultures. With other sources of carbon present amygdalin is more or less protected from the action of fungi. The influence of other factors, such as light, metal salts, and osmotic pressure on the hydrolysis of amygdalin by fungi is also considered. Quantitative data are entirely absent from the paper.

In a short paper HÉRISSEY and LEBAS<sup>9</sup> report that aucubine, the glucoside of *Aucuba Japonica*, is hydrolyzed by *Aspergillus niger* in acid solution and more slowly in neutral solution. Fuller details as to the mode of hydrolysis are lacking.

KNUDSON<sup>10</sup> reports the results of a series of investigations on the hydrolysis of tannic acid by fungi. He finds that the substance is generally toxic in concentrations of 2-5 per cent. Of 23 species of fungi, representing mucors, ascomycetes, and basidiomycetes, only 3, *Aspergillus niger*, *A. flavus*, and a species of *Penicillium*, made good growth, on a 10 per cent solution. *Aspergillus niger* was originally described from material growing on tannic acid solution by VAN TIEGHEM, who first observed that this organism and a species of *Penicillium* ("P. glaucum") brought about the hydrolysis of tannin with the formation of gallic acid. KNUDSON studied the action of *Aspergillus niger* and a species of *Penicillium* probably different from that of VAN TIEGHEM, and finds that the *Aspergillus* is the more active in bringing about the hydrolysis of tannic acid. A part of the gallic acid formed is consumed by the fungi. The extent to which the acid is consumed depends on the presence of other more favorable food substances. In a synthetic culture solution containing tannic acid the gallic acid is consumed to a greater extent than in gall-nut infusions which contain other food substances. In a 10 per cent sugar solution the gallic acid is left intact. The hydrolysis takes place also under anaerobic conditions, and under both aerobic and anaerobic conditions a part of the enzyme diffuses into the culture medium.

Statements by PRINGSHEIM and ZEMPLÈN that certain fungi (*Rhizopus tonkinensis*, *Mucor javanicus*, *Penicillium purpurogenum*, *P. africanum*, and *P. brevicaulis*) not possessing invertase are capable of utilizing cane sugar have led RITTER to reinvestigate the behavior of a number of fungi in relation to their capacity for utilizing that sugar. His results are in accord with the general proposition that in fungi, as well as in higher plants and in animals, cane sugar is not capable of direct assimilation, but can be utilized only by those organisms producing invertase. Among the fungi which RITTER<sup>11</sup> tested, *Mucor spinosus*, *M. javanicus*, *Thamnidium elegans*, *Rhizopus nigricans*, and *R. tonkinensis*

<sup>9</sup> HÉRISSEY, H., et LEBAS, C., Utilization de l'aucubine par l'*Aspergillus niger* v. Tgh. Compt. Rend. Soc. Biol. Paris 70:846-848. 1911.

<sup>10</sup> KNUDSON, L., Tannic acid fermentation I. Jour. Biol. Chem. 24:159-184. 1913.

<sup>11</sup> RITTER, G. E., Über das Verhältnis der Schimmelpilze zum Rohrzuoker. Biochem. Zeitschr. 42:1-6. 1912.

made practically no growth on nutrient solutions containing cane sugar with potassium nitrate or ammonium tartrate as sources of nitrogen. *Penicillium purpurogenum*, which grew well on cane sugar, was found to contain invertase, contrary to the statement of PRINGSHEIM and ZEMPLÉN.

JEGOROFF,<sup>12</sup> studying the assimilability of phytin by mold fungi (*Aspergillus niger* and *Penicillium* sp.), confirms the results of DOX, according to which that substance is hydrolyzed by the action of molds, the phosphorous radicle being utilized as a source of phosphorus by the fungi. Sterilization of nutrient solutions containing phytin does not in itself cause hydrolysis of the substance. In cultures containing peptone and cane sugar inorganic phosphorus (potassium dihydrogen phosphate) gave a better yield than phytin, but in cultures containing only cane sugar or only glycerine as sources of carbon, phytin gave better yields than inorganic phosphorus. Peptone gave low yields with both phytin and potassium dihydrogen phosphate, the culture showing little difference in favor of either.

The work of a number of investigators has shown that urea, uric acid, hippuric acid, and glycocoll serve not only as sources of carbon and nitrogen for fungi, but also as sources of nitrogen for green plants. The availability of these compounds as nutrients for fungi has been further investigated by KOSSOWICZ<sup>13</sup> with reference to the following fungi: *Botrytis Bassiana*, *Penicillium crustaceum*, *P. brevicaulis*, *Mucor Boidin*, *Cladosporium herbarum*, *Phytophthora infestans*, *Aspergillus glaucus*, *A. niger*, *Isaria farinosa*, and a species of *Fusisporium*. He finds that in cultures with cane sugar all the fungi made good growth in solutions containing urea or uric acid; *Cladosporium herbarum* failed to grow on solutions containing glycocoll; and *Penicillium crustaceum*, *P. brevicaulis*, *Aspergillus glaucus*, and *Cladosporium herbarum* failed to grow on hippuric acid. In later experiments, however, in which dextrose or mannite were substituted for cane sugar, and the culture solution was somewhat modified as to its inorganic constituents, these four species also were able to utilize all of the compounds in question. The cause of this peculiar difference of behavior is not discussed. Some of these compounds served as sources of both carbon and nitrogen for certain of the fungi. Uric acid served thus for *Aspergillus glaucus*, *Isaria farinosa*, *Penicillium glaucum*, *Mucor Boidin*, *Phytophthora infestans*, and *Botrytis Bassiana*; hippuric acid for *P. glaucum*, *A. niger*, *A. glaucus*, *I. farinosa*, *B. Bassiana*, *Ph. infestans*, *Cladosporium herbarum*, and *Fusisporium*; and glycocoll for *P. glaucum*, *C. herbarum*, *B. Bassiana*, *I. farinosa*, *Ph. infestans*, *A. niger*, *A. glaucus*, and *M. Boidin*.

<sup>12</sup> JEGOROFF, M. A., Über das Verhalten von Schimmelpilzen (*Aspergillus niger* und *Penicillium crustaceum*) zum Phytin. Zeitschr. Physiol. Chem. 82:231-242. 1912.

<sup>13</sup> KOSSOWICZ, A., Die Zersetzung von Harnstoff, Harnsäure, Hippursäure, und Glykokoll durch Schimmelpilze. Zeitschr. Gärungsphysiol. 1:60-62. 1912; also 2:51-54. 1912.



In connection with these experiments KOSSOWICZ<sup>14</sup> has examined the action of the extracts of six of the species of fungi on uric acid and on hippuric acid. He confirms the results of earlier investigators who have shown that the fermentation of hippuric acid is an enzymatic process, and shows also that the decomposition of uric acid by fungi is a process of similar nature. Extracts of *Aspergillus niger*, *Mucor Boidin*, *Phytophthora infestans*, *Isaria farinosa*, and *Botrytis Bassiana* were found to ferment both acids, while extracts of *Cladosporium herbarum* acted only on uric acid.

The acceptance of the liberation of ammonia as a criterion of the fermentation of hippuric acid has been criticized by DOX and NEDIG,<sup>15</sup> who find as a result of quantitative determinations of the glycocholl produced in the process that ammonia is formed only in small quantities as a result of secondary decomposition of glycocholl.

The mode of absorption and utilization of fats by fungi has been investigated by SPIECKERMANN.<sup>16</sup> By means of cultures on infusorial earth, from which the residual acids could be extracted, he was able to show that the higher fatty acids in the form of their salts (soaps, especially the calcium and the ammonium soaps whose decomposition does not result in the accumulation of strong alkali in the cultures) are utilized to a large extent. The somewhat contrary results of SCHMIDT are to be ascribed to his use of alkali soaps whose decomposition caused too great a degree of alkalinity of the culture medium. In cultures on agar plates in which sodium nitrate was present and in which the free fatty acids were distributed in a finely divided state throughout the agar, a broad clear zone, indicating the formation of soap by the action of the alkali set free by the utilization of the nitrate group, appeared around the colonies. When ammonium sulphate was used instead of sodium nitrate a narrower zone appeared about the colonies, showing that even in the acid medium an extracellular solution of the fatty acids takes place, although the process here is not as readily explained as in the first case. Similar phenomena observed with reference to fats show that they also undergo extracellular solution before being absorbed by living cells. From these observations the author concludes that fats are taken into the cell in the form of soaps or as free fatty acids.

As a result of an examination of a large number of yeasts and other budding fungi, as well as a number of filamentous forms found in connection with the industries dependent on fermentation, LINDNER and CZISER<sup>17</sup> find that the

<sup>14</sup> KOSSOWICZ, A., Die enzymatische Natur der Harnsäure- und Hippursäure-Gärung. Zeitschr. Gärungsphysiol. 1:121-123. 1912; also 1:317-319. 1912.

<sup>15</sup> DOX, A. W., and NEDIG, R. E., Enzymatische Spaltung von Hippursäure durch Schimmelpilze. Zeitschr. Physiol. Chem. 85:68-71. 1913.

<sup>16</sup> SPIECKERMANN, A., Die Zersetzung der Fette durch höhere Pilze. I. Der Abbau des Glycerins und die Aufnahme der Fette in die Pilzzelle. Zeitschr. Unters. Nahrungs- und Genussmittel. 23:305-331. pls. 3. 1912.

<sup>17</sup> LINDNER, P., und CZISER, S., Der Alkohol, ein mehr oder weniger ausgezeichneter Nährstoff für verschiedene Pilze. Wochenschr. Brauerei 29:1-6. 1912.

ability to assimilate alcohol, although varying with different races, is almost universal among these organisms. The loss thus sustained in the process of fermentation they suggest should be taken into consideration in devising methods of operation.

Similarly, in a short note WILL and HEUSS<sup>18</sup> show that, as is well known for other fungi, various budding forms, including species of *Mycoderma*, *Torula*, *Willia*, and *Pichia*, consume ethyl acetate, apparently utilizing both the acid and the alcohol radicle in their metabolism.

The study of the production of toxic substances by fungi growing on food products destined for human consumption has recently attracted much attention, especially in Italy in connection with the investigation of pellagra. From this point of view ALSBERG and BLACK<sup>19</sup> have investigated substances elaborated by two species of molds; the one, *Penicillium puberulum* Bainier, isolated from spoiled maize in Nebraska, and the other, *P. stoloniferum* from similar material from Italy. From the culture medium (RAULIN's solution) in which *P. puberulum* was grown, the authors isolated a substance to which the name penicillic acid, with the formula  $C_8H_{10}O_4$ , was given. The substance, which behaves like a monobasic acid, is fatal to animals when injected subcutaneously in doses of 0.2–0.3 grams per kilogram of body weight. It is formed more abundantly when the air supply of the fungus is limited than with full aeration. An acid medium also favors its production.

*Penicillium stoloniferum* elaborates a non-toxic substance to which the name mycophenolic acid is given. Its empirical formula is  $C_{17}H_{20}O_6$ . It behaves like a dibasic acid and resembles the lichen acids in many ways. With ferric chloride this substance gives the violet color of GOSIO's phenol reaction, which in Italy is regarded as a reliable test for the detection of deterioration in maize, but which the authors were unable to obtain in its characteristic form from samples of spoiled American maize.

In conclusion, the authors point out the desirability of utilizing biochemical behavior as an aid in the separation of species of molds which are not easily distinguished by morphological characteristics.

Owing to the ability of some molds to complete their development with traces of certain universal elements so minute that they cannot be removed by chemical means, the study of the influence of such elements on spore-production presents difficulties which account for the variant results obtained by different and sometimes by the same investigators. Formerly SAUTON<sup>20</sup> ascribed to iron a special rôle in the spore-production of *Aspergillus niger*, but later he and

<sup>18</sup> WILL, H., und HEUSS, R., Essigsäureäthylester als Kohlenstoffquelle für Hefe und andere Sprosspilze. Zeitschr. Gesamte Brauwesen. 35:128–129. 1912.

<sup>19</sup> ALSBERG, C. L., and BLACK, O. F., Contributions to the study of maize deterioration. U. S. Dept. Agric., Bur. Plant Ind. Bull. 270. pp. 48. pl. 1. 1913.

<sup>20</sup> Rev. BOT. GAZ. 55:86. 1913.

JAVILLIER<sup>21</sup> negated this conclusion and attributed the lack of spore-formation in the absence of iron to the deleterious effect of zinc in RAULIN's solution, for they found that when both zinc and iron were absent spores were produced by the fungus. Meanwhile, BERTRAND<sup>22</sup> reported experiments which seemed to indicate that manganese played a special part in the spore-production of *Aspergillus niger*.

The question of the influence of various elements on the spore-production of molds has now been further investigated by SAUTON.<sup>23</sup> He finds that if *A. niger* is grown on RAULIN's solution to which iron in the form of ferric ammonium citrate has been added, no spores are produced, thus strengthening his conclusion that iron plays no special part in the spore-production of that fungus. If, however, iron sulphate is added to the solution, spores are produced. The difference in behavior of the two salts may be ascribed to the absence of manganese in the one and its presence in the other. The author finds, however, that in a solution without zinc, spores are produced in the absence of any detectable traces of iron and manganese, and concludes, therefore, that if these elements are necessary for *A. niger*, the requisite quantities are limited to such minute traces that they cannot be detected chemically. He finds that in this respect *A. fumigatus* is a more favorable object of experimentation. If of the two elements in question manganese alone is present in the culture solution, this fungus produces spores after 15 days, while with iron also present spores are produced on the third day. If manganese is absent no spores are formed. It appears, therefore, that for this mold both iron and manganese are necessary for spore-production. The influence of some of the other elements of RAULIN's solution on spore-production was also determined. In the absence of sulphur *A. niger* grows poorly but nevertheless produces spores. *A. fumigatus*, however, does not produce spores in the absence of that element. No spores are produced by either mold in the absence of potassium, but the addition or withdrawal of caesium and rubidium are without influence. In the absence of phosphorus no spores are formed, but it does not seem to be possible to exclude magnesium to such an extent as to inhibit spore-formation.

It is likely that this work would lead to more definite results if it were separated from the problem of the toxicity of zinc, which although placed by RAULIN among the elements of his culture solution is not a substance necessary in the metabolism of plants.

BERTRAND and JAVILLIER<sup>24</sup> have republished their experiments on the action

<sup>21</sup> Rev. Bot. Gaz. 55:88. 1913.

<sup>22</sup> Rev. Bot. Gaz. 55:89. 1913.

<sup>23</sup> SAUTON, B., Sur la sporulation de l'*Aspergillus niger* et de l'*Aspergillus fumigatus*. Ann. Inst. Pasteur 27:328-335. 1913.

<sup>24</sup> BERTRAND, Dr., et JAVILLIER, M., Action combinée du manganèse et du zinc sur le développement et la composition minérale de l'*Aspergillus niger*. Ann. Inst. Pasteur 26:515-521. 1912.

of zinc and manganese, the first publication of which has been reviewed in this journal.<sup>25</sup>

From recent work of RITTER<sup>26</sup> and of HAGEM,<sup>27</sup> as well as from scattered observations in the older literature, it appears that the power of using nitrogen in the form of nitrite is widespread among filamentous fungi. A further contribution to this subject is made by KOSSOWICZ,<sup>28</sup> who finds that *Botrytis Bassiana*, *Penicillium glaucum*, *P. brevicaulis*, *Mucor Boidin*, *Cladosporium herbarum*, *Phytophthora infestans*, *Aspergillus glaucus*, *A. niger*, *Isaria farinosa*, and a species of *Fusisporium* grow readily on synthetic culture solutions containing potassium as the sole source of nitrogen; and cane sugar, dextrose, or mannite as carbon compounds. The formation of ammonia could be definitely shown to occur only in mannite cultures of *Phytophthora infestans* and in those of *Fusisporium*. In cultures containing sugars the ammonia reaction of Nessler's reagent is not reliable on account of the similar reaction given by dextrose. It is erroneous, however, to conclude from the absence of the ammonia test that nitrites are not reduced to ammonia before being assimilated. The absence of ammonia merely shows that it is not produced in excess of the quantity used. In the absence of quantitative data showing the yields produced, the effect of nitrogenous substances in the tap water which the author used in his experiments cannot be easily estimated.

KOSSOWICZ and GRÖLLER<sup>29</sup> have investigated the value of sulphocyanates as sources of nitrogen, carbon, and sulphur for fungi. The same species of molds mentioned in the paper reviewed above were used in the experiments. It was found that in the absence of other nitrogenous compounds (except such as were introduced by means of the tap water and as impurities in the other compounds used) the fungi made a feeble growth which soon ceased in nutrient solutions containing ammonium, potassium, sodium, or iron salts of sulphocyanic acid. Traces of hydrogen sulphide were detected only in cultures of *Mucor Boidin* and occasionally in those of *Aspergillus niger* and *A. glaucus*. The conclusion that the fungi used in these experiments can obtain nitrogen from sulphocyanates would seem to need further support in view of the fact that in all cases there was a cessation of growth after a few days. The fungi made no growth when sulphocyanates were the sole source of carbon or of both carbon and nitrogen. A feeble growth appeared when the substance was the sole source of sulphur. Sulphocyanates seem to be only slightly toxic, although they cause a distinct depression of growth, yet even in a 10 per cent

<sup>25</sup> BOT. GAZ. 55:89. 1913.

<sup>26</sup> Rev. BOT. GAZ. 55:91. 1913.

<sup>27</sup> Rev. BOT. GAZ. 55:463. 1913.

<sup>28</sup> KOSSOWICZ, A., Nitritassimilation durch Schimmelpilze. Zeitschr. Gärungsphysiol. 2:55-58. 1912.

<sup>29</sup> KOSSOWICZ, A., und GRÖLLER, L. VON, Rhodanverbindungen (Schwefelcyanverbindungen) als Kohlenstoff, Stickstoff, und Schwefelquelle für Schimmelpilze, Sprosspilze (Hefen), und Bakterien. Zeitschr. Gärungsphysiol. 2:59-65. 1913.

solution growth is not entirely inhibited. The non-toxicity of sulphocyanates has also been observed by FERNBACH and in extremely dilute solutions by SAUTON.<sup>30</sup>

In a short note KOSSOWICZ and LOEW<sup>31</sup> report the availability of sodium thiosulphate as a nutrient for a number of yeasts and fungi. Hydrogen sulphide, free sulphur, and sulphuric acid are among the products resulting from the action of different organisms on the compound.—H. HASSELBRING.

**The leaf-bud of *Cordaites*.**—LIGNIER<sup>32</sup> has made an intimate study of the developing leaves of *Cordaites* from a portion of a silicified bud from the Stephanian of Grand' Croix (Loire). His piece of material was about 3 cm. long and in the matrix immediately around the bud proper and concentric with it had adult leaves identical in structure with *C. lingulatus* of RENAULT. He thinks it reasonable to suppose that these were borne on the same branch which bore the bud itself, especially since he found these leaves identical in structure with the outermost ones of the bud, with the exception of such differences as might be due to age. His conclusion that the bud is that of *C. lingulatus* seems beyond reasonable doubt.

In the young leaves the primordial strands are very small, the bast appearing to develop first as in living forms. In the older leaves the bast is almost always completely destroyed, so that practically nothing was seen of the secondary bast. The protoxylem and centripetal metaxylem develop early, with elements of typical form, ring, spiral, scalariform, and reticulate. The centrifugal wood comes later, appearing first in the region of the protoxylem. About the same time appear certain cells which LIGNIER designates "cellules diaphragmatiques," situated between the bundle sheath and either the body of the centripetal or the sides of the arc of centrifugal xylem. The region occupied by it may be quite extensive. LIGNIER has not compared the bundle with that of cycads, but similar cells do occur in the same region in the cycad bundle, especially lateral to the base of the centripetal xylem.

The sheath consists of several layers of large cells which differentiate early. The cells begin to thicken their walls when the xylem consists of only one or two tracheids. They are most abundant ordinarily at the sides of the bundle, and show certain resemblances to the xylem tissue, to which LIGNIER refers. Most, if not all, of their transverse walls are covered with "punctuations aréolées" and "serées," which are larger, however, and more irregular than those of the tracheary elements. Those on the longitudinal walls are even

<sup>30</sup> Rev. Bot. GAZ. 55:86. 1913.

<sup>31</sup> KOSSOWICZ, A., und LOEW, W., Vorläufige Mitteilung über das Verhalten von Hefen und Schimmelpilzen zu Natriumthiosulfat. Zeitschr. Gärungsphysiol. 2:78. 1913.

<sup>32</sup> LIGNIER, O., Différenciation des tissus dans le bourgeon végétatif du *Cordaites lingulatus* B. Ren. Ann. Sci. Nat. Bot. IX. 17:233-254. 1913.

more irregular. The character of these cells is also very similar to that in the cycads, where the sheath cells right and left of the centripetal xylem are similarly pitted. It is very different from that described by Dr. STOPES for *C. principalis*. She states that the sheath of this form is composed of two parts, an inner of long and slender elements and an outer of short and large ones, both with bordered pits.<sup>33</sup> Recently Miss BENSON<sup>34</sup> has described a form (*C. Felicis*), from the Lower Coal measures of England, with a much less definitely differentiated inner sheath. LIGNIER considers that the "bois diaphragmatique" which he has described corresponds to this "inner sheath" in both these forms. If this conclusion is correct, and there seems little doubt that it is, then his form and that of Miss BENSON have less specialized and more cycadean sheaths than that of *C. principalis*. The bundles of these two forms would thus stand nearer to the *Poroxylon* type, of the detailed structure of which SCOTT in his *Studies in fossil botany* (p. 508) says it is "in fact, that of a cycad." It is interesting to note, in passing, that JEFFREY has chosen the specialized *principalis* type for comparison with *Prepinus* and the Abietineae, while the other is in substantial agreement with the araucarian leaf bundle.

LIGNIER describes at some length the differentiation of certain glandular cells within the bundle sheath. The sclerenchymatous strands above and below the bundle differentiate early, and the mesophyll as well. The cells of the latter have an abundant "protoplasme chlorophyllien probablement accompagné d'hydrates de carbone." The palisade consists ultimately of two or three layers of cells. The epidermis was very poorly preserved and afforded no new data. The bundles were seen to dichotomize, as in ordinary cordaitan leaves, though LIGNIER says that the division does not occur in the same way as STOPES has described in the case of *C. principalis*.

The type of leaf LIGNIER has described is very similar to that of the living broad-leaved forms of the Araucarineae.—R. B. THOMSON.

**Water requirement of plants.**—BRIGGS and SHANTZ<sup>35</sup> have conducted a series of experiments upon the water requirements of certain crop plants and obtained results which are important not only in determining the most economical plants to cultivate in semi-arid regions, but also in indicating a profitable line of purely ecological research with the natural vegetation of various habitats. The term "water requirement" indicates the ratio of the weight of water absorbed by a plant during its growth to the dry matter produced. The exhaustive review of the literature of the subject will be of great service to all interested in this and allied problems, and demonstrates the fact that while a considerable number of experiments have been performed by a number of

<sup>33</sup> New Phytol. 2: pl. 9, fig. 6. 1903.

<sup>34</sup> Ann. Botany 26:201-207. 1912.

<sup>35</sup> BRIGGS, L. J., and SHANTZ, H. L., The water requirements of plants. I. Investigations in the great plains in 1910 and 1911. II. A review of the literature. U.S. Dept. Agric., Bur. Plant Ind. Bulls. 284 and 285. pp. 49 and 96. 1913.

workers, the methods employed have as a rule been crude and inexact, leading to much uncertainty in the results. Still it is evident that there was an increase in the water requirement when the soil moisture content approached either extreme; when the soil was deficient in any plant food element; when the amount of soil used in the experiment was small; and when shading of the plants occurred. Atmospheric conditions profoundly affected the water requirement, being greater in dry than in moist air.

The extensive experiments of the present investigators in 1910 and 1911 were conducted at Akron, Colo., with plants grown in water-tight pots containing about 115 kilos of soil each, so sealed that the loss of water was limited to that resulting from the transpiration of the plants, water being added as required. Among other results, it was found that wheat consumed an average of 507 kilos of water for each kilo of dry weight produced, and taking this as the standard (100), the relative water requirements of certain other plants were as follows: alfalfa 211; rye 143; oats 122; barley 106; potato 88; maize 73; sorghum 60; millet 54; and such weeds as *Amaranthus retroflexus* and *Salsola pestifer* 63. These results would indicate the great suitability of sorghum and millet for semi-arid regions. The bulletins contain a mass of detail and much additional data valuable to students of the agriculture and ecology of the great plains.—GEO. D. FULLER.

**Agriculture on acid lands.**—It has long been known that moors and heaths have acid soil, and many ecological classifications, such as that of WARMING, regard acidity as the chief determining factor of the vegetation. In several interesting and important papers, COVILLE<sup>36</sup> has given the results of some experiments that should almost revolutionize certain phases of agricultural practice. As is well known, various species of *Vaccinium* and *Gaylussacia* are commonly sold in the markets as blueberries and huckleberries, and yet are not cultivated, as are most other commercial fruits. Many attempts have been made to cultivate these berries, and their failure is attributed by COVILLE to the fact that their cultivation has been attempted in rich garden soil. Ordinary cultivated plants, such as alfalfa or roses, grow well in rich garden soil and poorly in peat, unless the acidity of the latter is neutralized by lime. The blueberry, on the other hand, grows poorly in garden soil, thrives in peat, and grows poorly in peat neutralized by lime. After describing the root fungi and their probable rôle in making nitrogen available, the author gives directions for germinating and growing blueberries, showing that fruiting plants can be

<sup>36</sup> COVILLE, F. V., Experiments in blueberry culture. U.S. Bur. Plant Ind. Bull. 193. pp. 100. pls. 18. figs. 31. 1910.

———, The formation of leaf mold. Jour. Wash. Acad. Sci. 3:77-89. 1913.

———, Directions for blueberry culture. U.S. Dept. of Agric. Circular 122. pp. 11. 1913.

———, The agricultural utilization of acid lands by means of acid-tolerant crops. U.S. Dept. of Agric. Bull. 6. pp. 13. 1913.

had in a year or two from seed. It is suggested, however, that cuttings will generally prove more desirable. Of much importance is the fact that in COVILLE's experiments there were produced berries much beyond the usual size. The experiments were made chiefly on *Vaccinium corymbosum*, but it is believed that other species would show similar behavior.

In one of the later papers, COVILLE describes the changes in the formation of leaf mold, noting its early acidity and its subsequent alkalinity; whereas, peat (not only lowland peat, but also upland peat, as he terms it) retains its acidity, because of the comparative suspension of decay. In another paper, he urges farmers to utilize some of their acid soils by the growth of acid-tolerant plants, and not universally to neutralize them by the use of lime. The work of COVILLE is an excellent illustration of the application of ecological methods to agriculture.—H. C. COWLES.

**Sexuality of Mucorales.**—In his studies on the sexuality of the Mucorales, BLAKESLEE observed that the sporangium originating from the zygospore of *Phycomyces nitens* contains spores which with respect to sexual differentiation are of three types, giving rise respectively to plus, minus, and neutral mycelia. To account for the occurrence of these three sexually differentiated strains, BURGEFF<sup>37</sup> has formulated a hypothesis according to which the nature of the mycelium is determined by the nuclei which it contains. These may be either (+) or (−), and, since the spores contain one or more nuclei, it is evident that a particular spore may contain either all (+), or all (−), or both (+) and (−) nuclei. In the first two cases, the spores and the mycelium which they produce are said to be "homokaryotic," and in the other case "heterokaryotic." This hypothesis he tested in an ingenious manner. By inserting the tip of a young sporangiophore into the cut basal end of another of the opposite strain, and applying pressure to the wall of the outer one, he was able to rupture the tip of the inner hypha and bring about a mixture of the two masses of protoplasm with their respective (+) and (−) nuclei. Cultures from the sporangia produced by this "mixochimaera" gave (+), (−), and neutral mycelia.

He describes an analogous case of heterokaryosis presented by *Phycomyces nitens* var. *piloboloides*, which differs from the parent in the form of its sporangiophores. The spores of this variety give rise to mycelia which produce either *nitens* sporangiophores only, or *piloboloides* sporangiophores only, or both kinds mixed on the same mycelium. The homokaryotic types remain pure, but the mixed type continues to split in the manner described. Mixochimaeras, formed from the pure selected types, give rise to all three forms. Crosses of *P. nitens* with rigidly selected strains of the variety give rise to all possible forms of both (+) and (−) strains of both types and to heterokaryotic combinations. The production by means of the cross of a (−) strain of var. *piloboloides*, which is (+), is of special interest.—H. HASSELBRING.

<sup>37</sup> BURGEFF, H., Über Sexualität, Variabilität, und Vererbung bei *Phycomyces nitens*. Ber. Deutsch. Bot. Gesells. 30:679-685. 1913.



**Somatic chromosomes in *Vicia*.**—For several years the structure of chromatin has received intensive study at the laboratory in Louvain, and numerous papers have been published by GRÉGOIRE and his pupils. A recent paper by SHARP<sup>38</sup> deals with the somatic chromosomes of *Vicia Faba*. The most important feature of this paper is the investigation of the resting nucleus and very early prophase. Even those investigators who believe in the individuality of the chromosome generally admit that they cannot find the individual chromosome in the resting reticulum, but SHARP claims, apparently with good reason, that the chromosomes in many cases can be identified even in the resting nucleus. Some of the stages leading up to the splitting of the chromosome have been misinterpreted by previous investigators and important stages have been overlooked. The telophasic vacuolization of the chromosome, now found by everyone, often gives the impression of a longitudinal splitting, and such a splitting is frequently described; sometimes the vacuolization results in the formation of short spirals. By schematizing features like these, it is easy to fall into error. Whether the vacuolization has resulted in the simulation of a split thread, or in a spiral, SHARP finds that in prophase a simple zigzag thread is formed which gradually straightens and by axial vacuolization becomes split into the future chromosomes. Thus the actual splitting is later than some have supposed. The vacuolization which appears in telophase has nothing to do with any splitting of chromosomes. There are no chromomeres and no continuous spirems either at prophase or telophase.

The paper is another strong argument in favor of the theory of the individuality of the chromosome.—CHARLES J. CHAMBERLAIN.

**Phylogeny of Filicales.**—In continuation of his studies of the phylogeny of ferns, BOWER<sup>39</sup> has investigated the monotypic tropical American genus *Metaxya*, which is better known as *Alsophila blechnoides*. Since this fern "has suffered vicissitudes of terminology," having been referred to *Polypodium*, *Aspidium*, *Alsophila*, and *Amphidesmium*, the suggestion was natural that it might be a synthetic type. BOWER concludes that the species deserves to represent a distinct genus, and that it is phyletically in a more primitive position than the true Cyatheae. He has also included with this study a general survey of other relatively primitive and related genera. In connection with this comparative study, BOWER has attempted to estimate the value of various characters for phyletic purposes; and especially "to see whether the position which the sorus holds relative to the margin of the sporophyll is not a more reliable feature, in ferns, at large, than it has commonly been held to be." The conclusion of the whole matter is that, "so far as the value of the general

<sup>38</sup> SHARP, LESTER W., Somatic chromosomes in *Vicia*. *La Cellule* 29:297-322. pls. 1, 2. 1913.

<sup>39</sup> BOWER, F. O., Studies in the phylogeny of Filicales. III. On *Metaxya* and certain other relatively primitive ferns. *Ann. Botany* 27:443-447. pls. 32-34. 1913.

phyletic characters of the ferns can be estimated, the criterion of position of the nascent sorus may be held to take precedence, in point of early origin and constancy, over any soral characters except the primal features of the sporangium itself, and over any anatomical characters of the axis derivative from the protostele." This is certainly an important conclusion, and in accordance with it, the leptosporangiate ferns (exclusive of the Osmundaceae) are grouped into two series: the "Superficiales," in which the origin of the sorus is constantly from the leaf-surface; and the "Marginales," in which it is as constantly from the margin.—J. M. C.

**Morphology of *Riccia*.**—Miss BLACK<sup>40</sup> in a recent study of *Riccia Frostii* Aust. finds that this species is strictly dioicous and that the sex organs are scattered irregularly in acropetal succession. From the standpoint of the arrangement of sex organs, this indicates that *R. Frostii* is more primitive than *R. natans*, in which the antheridia are clustered in a disk, and the archegonia, which appear later, are usually in two rows. From the standpoint of restriction of antheridia and archegonia to different individuals, an advance beyond *R. natans* is clearly indicated.

Miss BLACK agrees with Miss HIRSH,<sup>41</sup> who also studied *R. Frostii*, that the air chambers are not produced by splitting of cell walls at the angles of the cells, but by papillate outgrowth. Unfortunately, the figure given, as was the case in the work of Miss HIRSH, does not show the earliest stage in the development of the chamber, but can as easily be cited as proof that air chambers arise by splitting at the angles of the cells of the dorsal layer. The youngest air chamber shown is too old to settle the question either way, but a study of the relation of cells in the figure indicates that possibly the chamber may have arisen by splitting of the dorsal layer. This splitting need not originally occur within the tissues as some recent writers assume, but may, as DEUTSCH showed in *Targionia*, extend from the surface inward.

The rest of the investigation, which includes the development of sex organs, spermatogenesis, and sporogenesis, gives us nothing new.—W. J. G. LAND.

**Peripheral leaf cells.**—In many leafy liverworts there is a marked difference in form and markings of the peripheral cells of the leaf as compared with those farther away from the edge. GARJEANNE,<sup>42</sup> as the result of a study of 10 genera, finds that the thickening of the peripheral cells is stronger if the plant is exposed to conditions which give great variation of water content;

<sup>40</sup> BLACK, CAROLINE A., The morphology of *Riccia Frostii* Aust. Ann. Botany 27:511-532. pls. 37, 38. 1913.

<sup>41</sup> HIRSH, PAULINE E., The development of the air chambers of Ricciaceae. Bull. Torr. Bot. Club 27:73-77. figs. 6. 1910.

<sup>42</sup> GARJEANNE, A. J. M., Die Randzellen einiger Jungermannienblätter. Flora 105:370-384. 1913.

that the peripheral cells, irrespective of their form and thickenings, show a smaller number of plastids and oil bodies than the other cells of the leaf; that they are frequently distinguished from the flat cells by a greater capacity for taking up aqueous methylene blue and other basic anilin dyes, as well as by a greater blackening with silver nitrate; that the cells which color most strongly are in general those from which regeneration shoots develop and that the greater capacity for taking up color is not due to tannin. He concludes that the peripheral cells contain materials which are of significance for the production of adventitious shoots; that transportation of this material is very possible and consequently any cell of the leaf may thereby become capable of regeneration.—W. J. G. LAND.

**The androecium of *Parnassia*.**—Mrs. ARBER<sup>43</sup> has made an anatomical investigation of the stamens of *Parnassia*, and has applied the results to the problem of the affinities of the genus. The vascular strands for the stamens arise at a lower level than those for the staminodia, and the two sets are independent. This seems to confirm the view that the staminodia represent an inner set of stamens. In *P. palustris* the vascular strand traversing the filament is mesarch, "and there are indications of numerous phloem groups arranged round the xylem." This is thought to mean the presence of vestigial vascular strands which indicate that each stamen of *Parnassia* is reduced from an ancestral fascicle of stamens, such as occurs in *Hypericum*. DRUDE's view that *Parnassia* deserves to represent a family of its own, related to Saxifragaceae, Droseraceae, and Hypericaceae, is confirmed, and the view is expressed that the affinity between *Parnassia* and the Saxifragaceae "has been somewhat overestimated."—J. M. C.

**The life history of *Thelygonum*.**—In a study of *Thelygonum* from the germination of the seed to the mature embryo, SCHNEIDER<sup>44</sup> deals with gross morphological features, the development of both staminate and ovulate flowers, the reduction divisions, the development of the gametophytes, fertilization, and the development of the embryo and seed coats. In the cytological portions of the paper, it is seen that the root tips have 20 chromosomes arranged in definite pairs, the reduced number is 10, and fertilization is of the usual double character. In conclusion, the author agrees with HALLIER in placing the Thelygonaceae near the Haloragidaceae. While there is still room for complete life history studies of new or unusual plants, in most cases the time has come for intensive work on special features. In this case it looks as if it might have been worth while to look for a differentiation among chromosomes.—C. J. CHAMBERLAIN.

<sup>43</sup> ARBER, AGNES, On the structure of the androecium in *Parnassia* and its bearing on the affinities of the genus. Ann. Botany 27:491-510. pl. 34. 1913.

<sup>44</sup> SCHNEIDER, HANS, Morphologische und entwicklungsgeschichtliche Untersuchungen an *Thelygonum Cynocrambe* L. Flora 116:1-41. figs. 23. 1913.

**Apical cells of Marsiliaceae.**—"Contributions to the developmental history of the Marsiliaceae" is the rather broad title of a paper by SCHNEIDER,<sup>45</sup> dealing with the apical cell and its segmentation in the vegetative organs of this family. The first part deals with the main axis and the second part with the lateral organs derived from it, the leaf, branch, and root. The apical cell of the axis is so oriented that two segments are dorsal and one is ventral. Roots are derived from the ventral segments, while leaves and branches come from the dorsal. In the behavior of the apical cells and their segments *Marsilia* and *Pilularia* differ from each other only in minor particulars. The work, which seems to be quite accurate, confirms and extends somewhat the earlier work of JOHNSON.—CHARLES J. CHAMBERLAIN.

**Anatomy of *Salicornia*.**—Miss DEFRAINE<sup>46</sup> has investigated the anatomy of *Salicornia*, and among the results the following are noted. The succulent "cortex" covering the internodes is phylogenetically derived from the basally developed leaf-sheath of the pair of leaves of the node above. The evidence for this seems quite convincing. The small, fleshy cotyledons fuse to form a cotyledonary sheath to the hypocotyl, similar to the leaf-sheath of the vegetative shoot. The occurrence of every transition between spiral cells and stereids led to the conclusion that the two are homologous structures, the former functioning chiefly in water storage, the latter in mechanical support. A peculiar kind of secondary growth sets in early in both root and stem.—J. M. C.

**Endogenous gemmae.**—The formation of endogenous gemmae is reported in *Haplozia caespiticia* by BUCH.<sup>47</sup> From within a gemma mother cell, numbers of which develop on the swollen end of a stem, two to four gemmae are produced and set free by the bursting of the wall of the mother cell. Mucilage within the mother cell absorbs water, swells, and bursts the wall. Endogenous gemmae have heretofore been reported for two genera, first in *Aneura* by GOEBEL and later in *Metzgeria* by EVANS.—W. J. G. LAND.

<sup>45</sup> SCHNEIDER, FRITZ, Beiträge zur Entwicklungsgeschichte der Marsiliaceen. Flora 105:347-369. fgs. 18. 1913.

<sup>46</sup> DEFRAINE, ETHEL, The anatomy of the genus *Salicornia*. Jour. Linn. Soc. Bot. 41:317-348. pls. 15, 16. 1913.

<sup>47</sup> BUCH, HANS, Über die Brutorgane der Lebermoose. 8vo. pp. ix+70. pls. 3. Helsingfors: J. Simellii Arvengars Boktryckeriaktiebolag. 1911.

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